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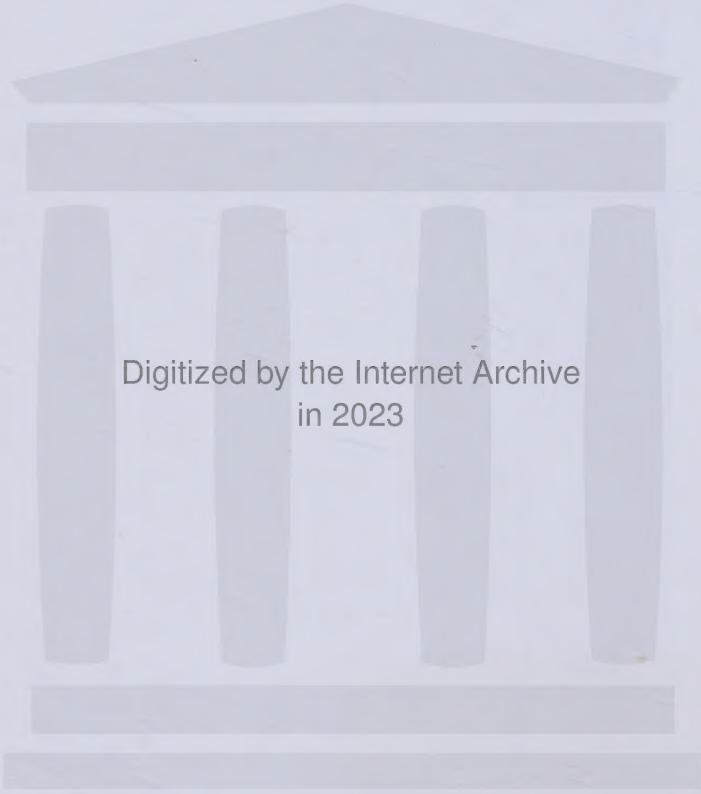
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SELECTION TRIAL DATA AS A BASIS FOR BREEDING AND SELECTION STRATEGIES

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ABSTRACT

Data from routine selection trials in four of the five selection stages of the Macknade breeding program are examined. Mean sugar content of the seedling populations, measured either as refractometer brix or CCS, is only slightly less than that of the major local cane variety Triton, even in previously unselected populations. Mean fiber content of those seedlings reaching the final stages of selection is only slightly higher than that of Triton and screening for fiber content in earlier stages of selection is not considered necessary.

Cane yield at all selection stages is markedly inferior to the yield of Triton, particularly in ratoon crops. The potential for selection gain for sugar content and cane yield is discussed and it is concluded that while considerable gains can be attained for cane yield, sugar content is probably close to the physiological limit. It is suggested that sugar content may receive undue emphasis in selection programs, as it generally shows a much higher degree of genetic determination than cane yield. Modifications to the Macknade breeding and selection program which give greater emphasis to cane yield are discussed.

INTRODUCTION

The effectiveness of a cane breeding program depends on many inter-related factors. The choice of parents and the combination of these parent clones in crosses is clearly fundamental to success (Bond[4], Roach[31]) but deficiencies in this respect can be at least partially offset by increased population size or more effective selection. Deficiencies in selection effectiveness can, in turn, be partially offset by decreased inter-generation interval or by introduction into the breeding cycle of superior genotypes from a separate breeding and selection program.

The difficulty of selecting superior genotypes from sugarcane progenies has long been recognized. In 1935 McIntosh[25] noted the effects of environment, genotype x environment interaction and competition on selection efficiency. In recent years these concepts have been defined in genetical and mathematical terms (Hogarth[18,19], Skinner[35]). Most emphasis in improving selection efficiency has

been devoted to the early, unreplicated stages of selection. The relative efficiency of bunch and single planting of seedlings has been examined (George[12], Herbert[17], Ojeda[28], Skinner[32,33,34]) and some researchers (Bond[3,4], Hogarth[19]) have suggested that family rather than individual selection may be more efficient in the genetic improvement of a population.

There have been numerous experiments aimed at assessing the effectiveness of selection for a particular character or set of characters, the correlations between such characters and prediction of response to selection (Brown[8], Cesnik and Vencovsky[11], Hogarth[20], Kadd *et al.*[21], Mariotti[23,24], Miller[26]). Direct measurement of many important characters of sugarcane is time consuming and expensive. Simple, indirect approximations are widely used, e.g. visual ratings for cane yield, refractometer brix for sugar content and rind hardness for fiber content. The combination of genetic data, economic data and indirect selection measures into a selection index has recently been reported by Miller *et al.*[27]

The effectiveness of a breeding and selection program can be monitored in several ways. One method, which has been used on several occasions at Macknade is to carry forward at each stage of selection a random sample of clones as a basis for measuring selection gain (Symington[36]). An alternative is to examine routine selection trial data in relation to existing commercial varieties and it is this rather simple approach which is used in this paper.

MATERIALS AND METHODS

An outline of the breeding and selection cycle which has been used at Macknade for several years is shown in Fig. 1. At each stage of selection the new seedling clones are compared with the most productive commercial varieties of the area. In the 7-year period for which results are analysed in this paper, Triton has been the major commercial variety. It has been included in all selection stages and thus, provides a standard of comparison for each population at each selection stage.

Stage 1 selection is a purely visual basis and only family ratings for general agronomic desirability (of which the principal component is cane yield), stalk thickness, tillering, stalk length and flowering percentage are retained. These data are used in between-family comparison rather than in comparison with the standard variety.

At Stage 2 only those clones which appear suitable for selection, based mainly on a visual estimate of cane yield, are brixed. They are selected if they are above a limit set by the mean and standard deviation for brix of plots of the standard variety. Brix data for Stage 2 are thus neither a complete nor a random sample of the whole cross. At subsequent stages, where the number of clones under tests is reduced, each individual clone in a cross is tested regardless of its apparent merit.

It may seem desirable in this type of comparison to follow the performance of several seedling series (populations of original seedlings) through the complete selection cycle. However, the length of the selection cycle (10 years if there are no

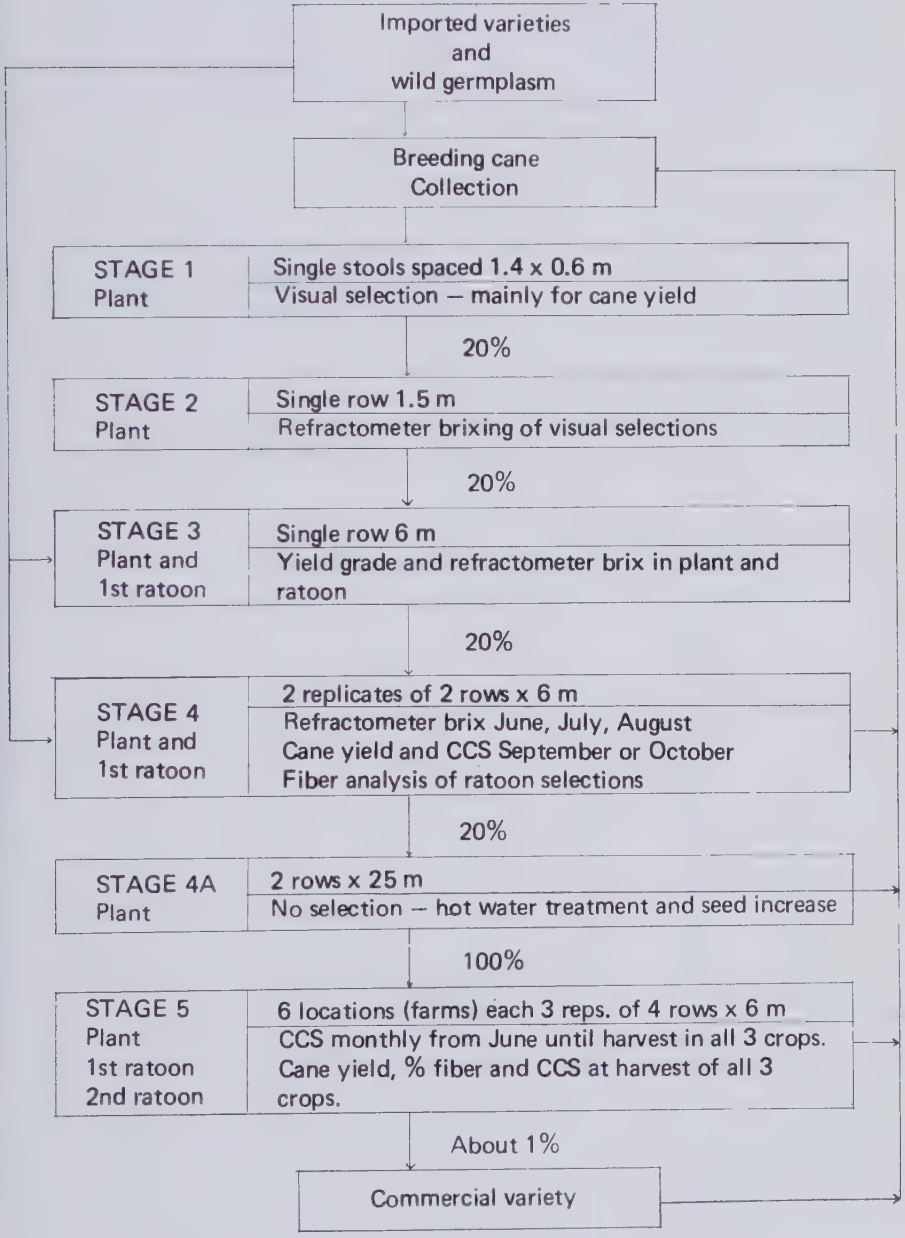

FIGURE 1. The Macknade breeding and selection program.

delays due to trial failures) precludes this. Triton, which is used as the standard in these comparisons, did not become the major district variety until 1972 and it is already being surpassed by the new Cassius variety. In addition, occasional changes are made to the selection system and the current program shown in Fig. 1 is the result of many modifications over the years. The procedure in this study has been to examine the results from several seedling series when tested in Stages 2-5 of the existing selection system.

RESULTS

Refractometer brix data for three series of Stage 2 selection trials are shown in Table 1. The 1973 series was not included, as excessive rainfall caused modification of the normal selection program for this series. The 520 crosses or families in these populations, although previously unselected for brix, averaged 96.4% of the mean brix of Triton. There are differences in performance between populations, e.g. the 1974 series averaged 101% of Triton and the 1975 series only 92% of Triton. There are also differences within sections of a series (1972 series in Table 1). Parentage of crosses in these groups does not differ significantly and the erectness ratings in Table 1 do not suggest different degrees of lodging as a cause of these

TABLE 1. Brix and erectness data for crosses in Stage 2 selection trials.

Series and year of selection	Trial block	No. of crosses	Refratometer brix			Erectness grade	
			Mean	C. V.	% of Triton	Mean	C. V.
1972 series	B7	53	19.37	10.0	93.8		
selected	B8	76	18.68	9.0	92.0		
1974	B9	40	20.58	6.3	99.0		
	B10	27	20.09	8.3	98.2		
1974 series	D9	90	21.63	6.5	102.8	4.69	17.9
selected	D11	74	22.34	5.5	99.8	4.54	18.9
1976	D12	42	21.31	8.5	100.8	4.25	23.5
1975 series	F3	48	21.02	6.0	91.9	4.66	14.2
selected	F4	52	20.42	5.9	92.4	4.68	19.2
1977	F5	18	20.94	3.2	92.8	4.291	29.8
Total and means		520	21.16		96.4		

Note: 1. C. V. = coefficient of variability

2. Erectness is graded on a scale 1 = completely erect to 9 = completely lodged.

differences. Seasonal and environmental effects are the most likely causes and these effects may be magnified by use of a single clone as the basis for measurement rather than a group of randomly selected clones or a group of standard commercial varieties (Pollock[29]).

Results for two seedling series in Stage 3 of the selection program are shown in Table 2. Following one screening for brix at Stage 2, the 2028 clones in this comparison averaged 97.2% of the brix value of Triton. The situation with yield grade, found in many comparisons to have a correlation of the order of 0.8 with actual yield, is very different. Plant crops have given only 71% and first ratoon crops only 62% of the mean yield grade of Triton.

Results for 744 clones tested in eight Stage 4 replicated trials are shown in Table 3. Average refractometer brix of the test clones is only slightly below the Triton mean (99.1%). CCS, a more exact measure of sugar content which takes account of non-sucrose components, averages over all trials 95.4% of the value of Triton. The lower percentage in the case of CCS may indicate a higher purity in Triton than the test clones, or it may simply be a reflection of the maturity curve for Triton, as CCS analyses are carried out late in the season, when Triton normally exhibits high CCS.

TABLE 2. Brix and yield grade data for clones in Stage 3 selections trials.

Series and year of selection	Trial and crop	No. of clones	Refractometer brix			Yield grade		
			Mean	C. V.	% of Triton	Mean	C. V.	% of Triton
1973 series	B9 – P1	244	23.85	4.65	99.0	4.88	24.2	65
selected	–1R		22.26	8.72	100.0	5.14	25.3	63
1977	B10 – P1	204	23.60	4.83	98.7	4.73	23.3	69
	– 1R		21.80	5.78	98.9	4.96	22.0	58
	B11– P1	90	22.97	6.97	94.9	4.54	21.8	81
	– 1R		21.02	7.71	95.3	4.68	21.2	69
1974 series	D12 – P1	209	23.80	5.92	94.8	4.62	19.7	75
selected	– 1R		22.43	5.44	97.3	4.94	24.3	64
1978	D13 – P1	669	23.94	5.51	95.5	5.19	22.9	69
	– 1R		22.25	6.34	98.4	5.25	23.8	60
	F1 – P1	152	2320	5.34	96.3	4.66	27.9	71
	–1R		21.69	5.99	97.4	5.04	23.4	66
	F2 – P1	360	23.28	8.21	96.0	5.69	27.8	70
	– 1R		22.45	6.19	98.9	5.75	24.2	55
Total and means	– P1	2028	23.52		96.5	4.90		71.4
	– 1R		21.99		98.0	5.11		62.1

Note: Yield grade is rated on a scale 1 = best to 9 = worst.

TABLE 3. Results from eight Stage 4 replicated trials.

Seedling series	Trial, crop and number of clones		Refractometer brix Jun.	Jul.	Aug.	At harvest Sept.–Oct. Av.	CCS	TCH	% fiber
1972 and	D3	$\bar{x}$	18.9	21.0		19.9	15.7	78	
1971 series	P–1977	C.V.	7.1	5.7		5.6	7.6	24.1	
	150 clones	% T	100	99		100	90	78	
	D3	$\bar{x}$	19.1	20.6	21.6	20.4	15.9	83	13.5
	1R–1978	C.V.	7.8	7.2	7.5	6.5	6.5	19.6	11.9
	150 clones	% T	97	97	95	96	95	78	200
1972 and	C2	$\bar{x}$	18.7	20.5		19.6	15.9	78	
1971 series	P–1977	C.V.	8.4	7.6		7.6	6.9	40.0	
	78 clones	% T	103	98		100	94	60	
	C2	$\bar{x}$	18.4	20.2	21.5	20.0	15.0	74	12.9
	1R–1978	C.V.	7.0	6.5	5.6	5.5	7.9	23.3	13.9
	78 clones	% T	99	99	98	98	94	65	96
1972 and	B15	$\bar{x}$	19.8	21.5		20.6	16.1	91	
1971 series	P–1977	C.V.	6.2	5.3		5.4	6.5	24.9	
	89 clones	% T	101	100		101	96	64	
	B15	$\bar{x}$	19.1	20.5	22.0	20.5	15.8	86	12.8
	1R–1978	C.V.	7.9	6.6	4.7	5.6	8.0	24.1	10.1
	89 clones	%T	100	99	99	100	99	77	101
1971 series	B11	$\bar{x}$	21.4	22.7	23.2	22.5	16.8	50	
	P–1976	C.V	4.1	3.6	3.9	3.5	5.3	32.8	
	60 clones	% T	102	101	101	102	97	62	
	B11	$\bar{x}$	19.5	21.7		20.6	15.9	60	13.2
	1R–1977	C.V.	6.5	5.5		5.5	6.1	20.1	9.6
	60 clones	% T	101	99		100	100	72	103
1970 series	B17	$\bar{x}$	21.6	22.6	23.6	22.6	15.7	45	
	P–1975	C.V.	4.7	4.8	4.3	4.1	7.5	33.8	
	31 clones	%T	101	97	100	99	94	79	
	B17	$\bar{x}$	21.3	22.2		21.8			
	1R–1976	C.V.	4.5	5.1		4.5			
	31 clones	%T	94	95		95			

TABLE 3. (cont'd)

Seedling series	Trial, crop and number of clones		Refractometer brix Jun.	Jul.	Aug.	At harvest Sept.—Oct. Av.	CCS	TCH	% fiber
1970 series	D4	$\bar{x}$	20.1	22.2	22.5	21.6	15.7	50	
	P—1975	C.V.	6.8	4.8	4.0	4.5	5.5	20.2	
	80 clones	% T	98	100	100	99	96	66	
	D4	$\bar{x}$	21.5	22.2		21.9	16.5	52	14.7
	1R—1976	C.V.	4.1	4.2		3.9	5.0	18.8	8.6
	80 clones	% T	100	98		99	96	70	115
1970 series	D5	$\bar{x}$	19.1	20.8	21.7	20.5	14.7	57	
	P—1975	C.V.	8.2	6.6	5.2	5.9	7.8	25.6	
	128 clones	% T	102	99	98	100	93	75	
	D5	$\bar{x}$	19.8	21.5		20.7	16.4	69	13.2
	1R—1976	C.V.	6.3	4.9		5.4	6.0	23.2	7.3
	128 clones	% T	101	99		100	97	80	98
1970 series	D6	$\bar{x}$	20.6	22.2	22.3	21.7	15.6	51	
	P—1975	C.V.	.6.0	4.5	3.9	4.1	4.7	24.5	
	128 clones	%T	98	99	98	98	95	70	
	D6	$\bar{x}$	19.7	21.3		20.5	16.1	67	13.9
	1R—1976	C.V.	5.1	5.0	5.0	4.5	6.3	20.3	11.6
	128 clones	% T	99	96		98	94	90	111
% of Triton in — plant crops			100.6	99.1	99.4	99.9	94.4	69.3	
	— ratoon crops		98.9	97.8	97.3	98.3	96.3	76.4	103.4

Note: 1. *x = mean of seedling clones in the trial*
2. *% T = mean of all seedling clones as a percentage of the mean of triton plots*
3. *TCG = tons of cane per hectare*
3. *TCG = tons of cane per hectare*

Ratoon cane yield and percentage fiber are only determined in Stage 4 for those clones which appear to have some prospect of selection on the basis of plant and ratoon crop results to that stage. Performance of this select group, comprising about 30% of the total is a little better for ratoon yield (76% of Triton) than plant yield performance (69% of Triton). Generally, the test clones are only a little above the fiber level of Triton (103.4%), which suggests that prior screening for fiber content by rind hardness testing or actual fiber determination would be of doubtful value. Testing for fiber content in Stage 4 seems justified before clones enter the extensively replicated and costly Stage 5 trials.

TABLE 4. CCS, cane yield and % fiber data for clones in plant (P) 1st ratoon (1R) and 2nd ratoon (2R) Stage 5 selection trials.

Series and number of clones	Crop and year		CCS Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Av.	Tons cane/ ha.	% fiber
1970 series	P	$\bar{x}$	12.1	13.9	14.7	15.2	15.0	15.7	14.4	97	12.8
61	1978	C.V.	10.8	7.8	6.0	6.4	6.7	5.0	5.8	14.8	8.6
clones		% T	108	102	97	94	96	98	98	75	99
1969 series	P	$\bar{x}$	13.3	15.3	15.9	16.3			15.2	77	12.5
37	1977	C.V.	8.5	6.8	5.0	4.9			5.6	14.0	9.8
clones		% T	94	97	96	96			96	75	102
	1R	$\bar{x}$	14.0	15.8	16.0	15.9	16.5	16.2	15.7	60	13.2
	1978	C.V.	7.6	4.9	4.9	4.5	5.6	5.1	4.5	23.6	9.1
		% T	99	98	96	96	98	98	97	68	106
1968 series	P	$\bar{x}$	14.9	15.3	15.7	16.1			15.51	85	12.5
68	1976	C.V.	6.6	5.8	5.0	5.0			5.0	13.2	9.7
clones		% T	103	105	103	101			103	72	103
	1R	$\bar{x}$	13.8	15.6	15.8	15.9	17.8		15.8	62	12.6
	1977	C. V.	6.7	5.6	5.1	4.8	5.0		4.4	19.8	9.4
		% T	100	99	98	96	100		99	62	99
	2R	$\bar{x}$	13.1	15.3	15.2	16.3	16.7	14.8	15.1	54	13.2
	1978	C.V.	8.3	6.2	7.0	5.0	5.6	5.2	5.2	28.2	8.9
		% T	101	101	96	99	99	95	97	56	104
1967 series	P	$\bar{x}$	13.1	15.2	14.4	15.4	15.5	14.4	14.7	53	13.2
22	1975	C.V.	8.3	7.0	6.8	4.9	8.5	8.1	6.0	27.3	6.1
clones		% T	94	94	95	96	99	106	97	80	102
	1R	$\bar{x}$	12.4			16.5			14.5	32	13.3
	1976	C.V.	16.6			7.9			10.3	35.9	6.8
		% T	102			96			99	64	99
	2R	$\bar{x}$	13.3	15.7					14.5	31	
	1977	C.V.	12.0	8.5					9.6	38.3	
		% T	99	93					95	62	

TABLE 4 (contd)

Series and number of clones	Crop and year		CCS Jun	Jul.	Aug.	Sep.	Oct.	Nov.	Av.	Tons cane/ ha.	% fiber
1966 series	p	$\bar{x}$	12.2	13.4	14.1	14.8	15.0		13.9	79	12.9
47	1974	C.V.	10.9	9.9	9.7	8.4	6.9		8.4	14.8	9.8
clones		% T	103	99	96	99	98		99	83	105
	1R	$\bar{x}$	12.2	13.7	13.9	15.0	14.4	13.3	13.8	66	13.2
	1975	C.V.	12.2	9.2	8.3	6.7	6.8	10.8	7.5	21.1	8.9
		% T	90	96	93	96	98	98	96	70	104
	2R	$\bar{x}$	12.9	12.6	13.7	15.2	17.0		14.6	60	12.7
	1976	C.V.	8.8	10.0	9.3	7.4	6.9		7.1	34.6	10.7
		% T	98	98	101	94	97		98	77	105
% of Triton in-plant			100	99	97	97	98	102	99	77	102.2
	–1R		98	98	96	97	99	98	98	66	102.0
	–2R		99	99	99	97	98	95	97	65	104.5

Clones in Stage 5 represent the end product of a breeding and selection system and deficiencies in these clones reflect deficiencies in this system. It is apparent from the data in Table 4 that cane yield, and particularly ratoon cane yield, is the greatest deficiency. Plant crops in Stage 5 have yielded only 77% of the yield of Triton, with first and second ratoon crops yielding only 66% and 65% respectively. Sugar content of clones in Stage 5, as in preceding stages, is on the average only a little below that of Triton. Recovery of clones with sugar content equal to or above that of the standard could be expected with reasonable frequency.

Of the populations detailed in Table 4, only the 1970 series was subject to screening for fiber content prior to testing in Stage 5, as this procedure has only recently been adopted. Since the average fiber content of the Stage 5 clones is only 102.5% of Triton, there is again little evidence to suggest a need for screening for fiber content prior to Stage 4.

DISCUSSION AND CONCLUSIONS

Cane yield and sugar content are the two basic selection criteria of any sugarcane breeding program. The importance of other criteria and the selection methods used to achieve them will depend on local industry requirements and the genetic material available in the breeding collection.

The problem of low cane yield or vigor in sugarcane seedling populations is not new, nor is it unique to the Macknade breeding program. In a symposium held in conjunction with the ISSCT 11th Congress, authors from Hawaii[38], Louisiana[5], Mauritius[13], South Africa[6] and Queensland[10] identified inferior cane

yield as the principal cause of discard of sugarcane seedlings. Bull and Glasziou[9] estimated maximum potential cane yield at 280 tons of cane per hectare for a 12 month period. There have been only isolated reports of cane yields of this order (Anon[1,2] George[12], and Ham[16]) and these yields have only been achieved in carefully maintained irrigated trials. F. W. Went, in correspondence with Mangelsdorf[22], suggested from physiological considerations a limiting concentration of about 25% sugars in the juice of a normally functioning sugarcane plant. Refractometer brixes in the range 25-27 degrees brix are frequently recorded in various selection stages and in the breeding collection at Macknade, particularly in clones of *S. officinarum.* It would seem that the highest levels of sucrose found in the best clones of *S. officinarum* and in the best of modern hybrids are at or near the physiological limit. Roach[13] concluded that the task of cane breeders in many countries in the future would be to retain sugar content at or near existing maximum levels while increasing cane yields, principally by selecting varieties better adapted to sub-optimal environments.

Skinner[35] gave the following definition of response to selection, which is appropriate for clonally propagated generations of sugarcane:

$$\Delta G = i\, g^2 \sigma p$$

where ΔG = gain from selection
i = selection difference in standard units of measurement
g^2 = heritability in the broad sense, repeatability or degree of genetic determination
σp = phenotypic standard deviation

Repeatability of sucrose content and its estimators (brix, pol, CCS etc.) and of cane yield and its components (tillering and stalk weight) has been estimated in many experiments (Brown *et al.*[7], Hogart[19], Symington[36]). Repeatability of sucrose content or its estimates has almost invariably been found to be higher than repeatability for cane yield. The general nature of this finding may have resulted in undue emphasis being placed on sugar content during selection in some breeding program. The fact that repeatability is only one of three components determining gain from selection may also have been overlooked, together with the fact that sucrose content generally shows much less phenotypic and genotypic variability than cane yield (Mariotti[23]). Walker[37], Watkins[39] and Hogarth[19] concluded on the basis of correlation and repeatability estimates alone that brix was a better selection criterion in early stages of selection than cane yield.

It appears from the routine selection trial results in this study and from specific selection methods trials at Macknade[36] that breeding and selection strategies need to be oriented towards increased cane yield particularly in ratoon crops. A program of incorporation of new germplasm into the breeding cycle has been in progress at Macknade since 1962[30]. Incorporation and evaluation of new genetic material in a breeding program is a slow process, but there are indications that this new germplasm is producing increased cane yields (Roach[31]).

The selection program shown in Fig. 1 is presently being modified to give greater emphasis to cane yield, particularly in ratoon crops. Actual cane yields will replace yield grading of ratoon crops of Stage 3. Selection from ratoon crops in early stages of selection is practised in many countries, e.g. Argentina (Mariotti[24]), Barbados and Guyana (Watkins[39]), Louisiana (Gaimalva *et al.*[14]), Teunion (Giroday[15]) and South Africa (Bond[4]). It has recently been incorporated into Stage 2 of the Macknade program and may need to be used further in other selection stages as an inexpensive means of eliminating clones which ultimately fail because of this defect.

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DATOS DE EXPERIMENTOS DE SELECCION COMO BASE PARA EL MEJORAMIENTO Y ESTRATEGIAS DE SELECCION

B. T. Roach

RESUMEN

Se examinan los datos provenientes de experimentos de seleccion de rutina en cuatro de los cinco estadios de seleccion en el programa de mejoramiento de Macknade. El contenido medio de azucar en las poblaciones de plantines, medidas ya sea con brix refractometrico o CCS, es apenas inferior al de la variedad de mayor difusion Triton, incluso en poblaciones sin seleccion previa. El contenido medio de fibra de los plantines que alcanzan los estadios finales de selecccion, es algo superior al de Triton, no considerandose necesario seleccionar por contenido de fibra en las primeras etapas de seleccion.

El rendimiento de caña en todos los estadios de seleccion es marcadamente inferior al de Triton, particularmente en las cañas socas. Se discute la ganancia por seleccion potential para contenido de azucar y rendimiento de caña concluyendose que mientras que es posible lograr considerables ganancias en rendimiento de caña, el contenido de azucar esta probablemente cerca de su limite fisiologico. Se sugiere que la riqueza en azucar puede estar siendo atendida en forma exagerada en los programas de seleccion, como consecuencia de que muestra un mayor grado de determinacion genetica que el rendimiento de caña. Se discuten algunas modificaciones al programa de seleccion en Macknade dirigidos a dar mayor enfasis a la seleccion por rendimiento de caña.

SELECTION INDICES FOR HIGH YIELD IN SUGARCANE

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ABSTRACT

Selection indices for high cane and sugar yields were determined from ten experiments for three cropping seasons.

The zero order correlation coefficient and the coefficient of determination indicated that high sugar yield was primarily due to tonnage.

Stalk weight and millable stalks per stool were closely associated with high tons cane per hectare and explained most of the variance in sugar yield among the tonnage yield components.

The most stable and desirable index for selection for high tons cane and sugar yields was more millable stalks per stool.

INTRODUCTION

Sugar yield and other economic traits are controlled by many genes. As quantitative characters, they are easily influenced by changes in the environment. For this reason, extra care should be exercised in picking out clones in the F1 population after hybridization so that selections are genetic rather than environmental variations.

The expression of a plant character may also be influenced by other traits. Quantitative estimates of these relationships may provide information useful in selection.

A more reliable selection criteria is necessary. The selection indices should not be only closely associated with high yield but it should also be easily distinguished visually, relatively stable among varieties and environmental conditions, and preferably pre-harvest characters. These selection indices could be obtained from my parent used in hybridization. According to Hebert[4], stalk diameter, erectness of stalk, stalk per stool and brix of progeny could be predicted from the characteristics of the parents.

Empig *et al.*[3] reported regression as a means of determining stability. If B = 0, it means that the genotype has little or no response to changing environment. The most desirable genotype is one with high mean and low "b" in tests in several locations.

In this study, the association of some characters with tonnage and the magnitude of their influence on sugar production were analyzed. The parameters of stability of these characters as tonnage and sugar yield components and the influence of other traits and varieties from ten experiments for three cropping seasons were evaluated.

MATERIALS AND METHODS

Widely grown varieties differing in yield components were used. Phil 56226, high in picul sugar per ton cane (PS/TC) and medium in tonnage, and Phil 6007, high in tons cane per hectare (TC/Ha) and medium in sugar quality were planted 33 cm in rows one meter apart. The varieties were distributed at random in plots five x five meters. The plots were fertilized with 200-100-350 kilograms per hectare of nitrogen, phosphorous and potassium, respectively, at planting.

All data were taken at harvest. The stalks in the inner three rows and 4.3 of each plot were harvested for yield. Fifteen stalks selected at random from each plot were used for juice analysis.

RESULTS AND DISCUSSION

The Relationship of Cane Yield and Sugar Quality with other Plant Characteristics

The varieties differed in their important sugar yield components. Phil 56226 was stable in sugar quality as indicated by low "b" and high mean. It produced significantly better PS/TC while Phil 6607 was outstanding in TC/Ha (Table 1).

TABLE 1. Means and regression values of some plant characters of two recommended sugarcane varieties

CHARACTERS	MEAN 1/		Regression Values 2/	
	Phil 56226	Phil 6607	Phil 56226	Phil 6607
1. Stalk diameter (cm)	2.62 b	2.98 a	19.62**	−59.48
2. Stalk length (cm)	257.15 b	287.58 a	0.64**	10.57
3. Millable stalk/stool	3.05 a	3.30 a	21.48**	35.51**
4. Stalk weight (kg)	1.01 b	1.20 a	59.59	138.28**
5. Picul sugar per ton cane	1.92 a	1.75 b	87.20	115.53
6. Tons cane per hectare	87.20 b	115.53 a	46.30**	74.81**
7. Piculs sugar per hectare	160.00 b	203.60 a		

1/ Means with similar letters between varieties are not significant at HSD 0.05
2/ Values within varieties with asterisks are significant at t.01.

High degree of interdependence existed between diameter, length, weight and number of millable stalks per stool with tons cane per hectare (Fig. 1). These were indicated by positive correlation coefficients, all significantly different from zero at one per cent level. Stalk weight which is a function of tonnage was more influenced by stalk length (0.47**) than by stalk diameter (–0.08).

Of these plant characters, stalk diameter (–0.40**) and stalk length (–0.44**) showed negative and significant influence on sugar quality. The greater negative influence of stalk length could be attributed to the presence of high reducing sugars in the top parts of the stalk which tend to lower sugar quality.

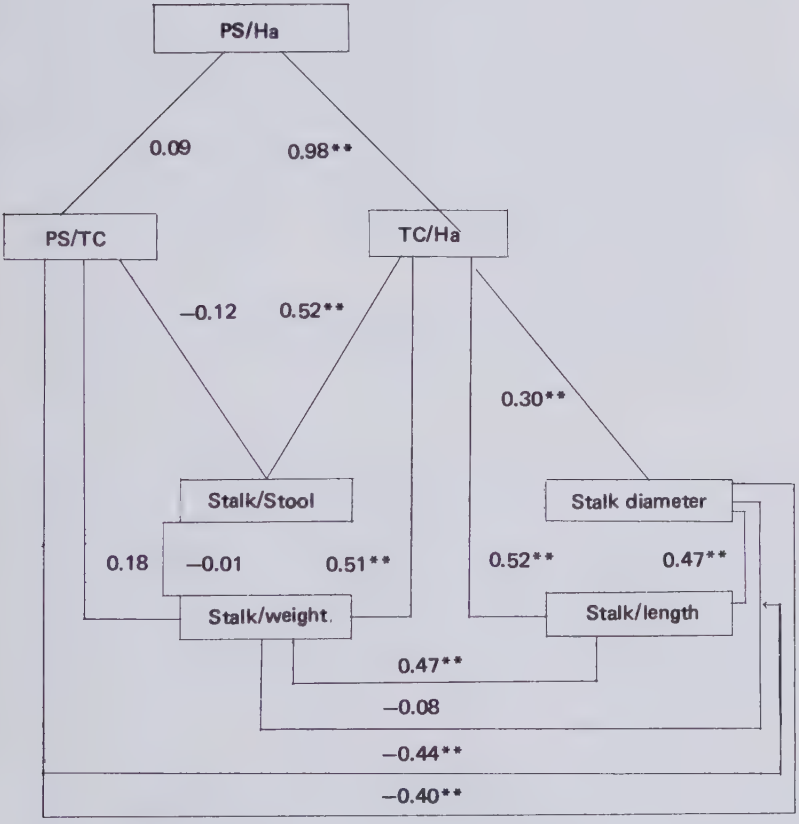

FIGURE 1. Relationship of sugar yield (PS/Ha) with sugar quality (PS/TC) and the components of cane yield (TC/Ha)

The Association of sugar yields with other plant characteristics

High sugar yield was mainly due to tonnage. This was probably because of the close association of sugar yield with stalk length, stalk weight and millable stalk per stool, plant characteristics which are all important components of tonnage. Similar observation was made by Dosado *et al*[1]. This suggests that selection indices for high sugar yield should be those characters which contribute directly to high tonnage. Maximizing sugar yield through genetic maniputation and fertilization to increase tonnage is easier to achieve (Empig[2]).

To further verify the importance of these and other characters as sugar yield components, coefficient of determination (R^2) were computed dropping one character at the time from the equation. The difference between the R^2 of the equation when the character was excluded from the total coefficient of determination was the contribution of the former to the variance in sugar yield.

As shown in Table 2, the R^2 values were .63 or 63%, .91 or 91%, 92 or 92%, .93 or 93%, and .95 or 95% when tons cane per hectare, piculs sugar per ton cane, weight of stalk and millable stalk per stool, length of stalks and diameter of stalks were dropped individually from the equations, respectively. These plant characters contributed 36%, 8%, 7%, 6% and 4%, respectively, in the variance in sugar yield. All these characters including PS/TC have an R^2 of .99 or 99%, considered of sufficient magnitude to explain the variance in sugar yield.

Selection Indices and Their Stability

As previously mentioned, stalk weight and number of millable stalk per stool were among the most important components of both cane and sugar yields and maybe used as indices for selection.

Close examination of the simple correlation coefficients show that stalk weight and millable stalk per stool were both independent of PS/TC. Their contribution to the total variance in sugar yield were similar. Furthermore, their values remained essentially the same in combination with other characters in the first and second order partial correlation after one or two traits were held constant or adjusted to the mean of the population (Table 3). Moreover, they also have slightly changed in combination with other tonnage yield components in the multiple regression equations. All these indicated their desirablity and stability as indices for high cane and sugar yields.

Of these two plant characters, the number of millable stalks per stool appeared to be a better index of selection. It is the most important tonnage yield component as indicated by the non-significance of the R-value when it was excluded from the multiple correlation analysis and is the only character which did not differ significantly between varieties. Selection for millable stalks per stool could easily be made and without necessarily harvesting the clone.

TABLE 2. Multiple regression equations estimating the sugar yield per hectare of sugarcane varieties

	Predicting Equations[1]	R^2	Standard Error of Estimate
1	$Y = -204.37 + 11.36x_1 + 6.15x_2 - 0.05x_3 + 7.45x_4 + 0.46x_5 + 0.55x_6$	.99	0.89
2.	$Y = -49.27 + 2.70x_1 + 3.09x_2 + 0.02x_3 + 8.77x_4 + 2.82x_5$	.63	1.50
3.	$Y = -63.08 - 7.68x_1 - 2.39x_2 + 0.10x_3 - 0.09x_4 + 0.56x_6$	.92	10.79
4.	$Y = -77.76 - 0.81x_1 - 0.90x_2 + 0.83x_3 - 0.36x_5 + 0.86x_6$	.92	9.24
5.	$Y = -28.89 - 2.39x_1 + 0.23x_2 + 7.59x_4 + 0.73x_5 + 0.58x_6$	.93	9.13
6.	$Y = -29.81 + 0.02x_1 + 0.03x_3 + 5.19x_4 + 2.26x_5 + 0.03x_6$	.95	6.12
7.	$Y = -83.65 + 2.22x_2 + 0.02x_3 + 7.94x_4 + 0.14x_5 + 0.47x_6$	.91	7.89

1/ x_1 *Picul sugar per ton cane*
x_2 *Diameter of stalk (cm)*
x_3 *Length of stalk (cm)*
x_4 *Number of millable stalk per stool*
x_5 *Weight of stalk (kg)*
x_6 *Tons cane per hectare*
Y *Yield index*

After selection of F1's with more millable stalks however, efforts should be directed in identifying clones with high brix among selected population. Rosario[5] pointed out that under certain set of conditions and input levels, maximum sugar yield is feasible when there is optimum contribution of both tonnage and picul sugar per ton cane.

TABLE 3. Simple, partial and multiple correlation coefficients of number of millable stalk per stool (X_1), stalk length (X_2), stalk weight (X_3), stalk diameter (X_4) and tons cane per hectare (X_5)

Zero Order Correlation		Partial Correlation First Order		Partial Correlation Second Order		Multiple Correlation			
Trait	Coef.	Trait	Coef.	Trait	Coef.	Trait	Coef.	Trait	Coef.
r_{51}	.52**	$r_{51.2}$	.53**	$r_{51.23}$	.44**	$R_{5.12}$	.48**	$R_{5.123}$	.59**
		$r_{51.3}$	.62**	$r_{51.24}$	.53**	$R_{5.13}$	.49**	$R_{5.124}$	.49**
		$r_{51.4}$	.52**	$r_{51.34}$	.71**	$R_{5.14}$	.32**	$R_{5.134}$	.80**
r_{52}	.52**	$r_{52.1}$	.53**	$r_{52.13}$	.34**	$R_{5.23}$	.36**	$R_{5.234}$	.29
		$r_{52.3}$	.36**	$r_{52.14}$	.48**	$R_{5.24}$	.27		
		$r_{52.4}$	.45**	$r_{52.34}$	.20	$R_{5.34}$	.37**		
r_{53}	.51**	$r_{53.1}$	.61**	$r_{53.12}$	.48**				
		$r_{53.2}$	.36**	$r_{53.14}$	.58**				
		$r_{53.4}$	.56**	$r_{53.24}$	.42**				
r_{54}	.30**	$r_{54.1}$	.26	$r_{54.12}$	.34**				
		$r_{54.2}$	.06	$r_{54.13}$	.66**				
		$r_{54.3}$	.39**	$r_{54.23}$	−.09				

* *Significant at 5% level*

** *Significant at 1% level*

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INDICES DE SELECCION PARA ALTOS RENDIMIENTOS EN CAÑA DE AZUCAR

V.G. Dosado, R.E. Tapay y H.M. Miayo

RESUMEN

Le determinaron indices de seleccion a partir de 10 experimentos durante tres cosechas. El coefficiente de correlacion de orden caro y el coefficiente de determinacion indicaron que los altos rendimientos de azucar se debieron primeramente al rendimiento agricola.

El peso de los tallos y los tallos molibles por planton estuvieron asociados fuertemente con altas toneladas de caña/hectarea y explicaron la mayor parte de la variación en rendimientos de azúcar entre las toneladas de los componentes del rendimiento.

El indice mas deseable y mas estable, para la seleccion de altos rendimientos de azucar y de caña fue tallos mobible por planton.

EARLY STAGES OF VARIETAL SELECTION PROGRAM IN FIJI

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ABSTRACT

A new scheme has been designed to select sugarcane varieties based on measurable characters during the early stages of the program. These were based on a single character specifications as demanded by the industry unlike the previous procedure where total performance was considered of greater value.

INTRODUCTION

The success of any breeding program is mainly dependent on the efficiency and effectiveness of the selection system geared to the requirements of the industry. Often it has been observed that many plant breeders set their goal to achieve improvement on single characters such as resistance to a specific disease, increase of sucrose concentration in juice, etc. Unfortunately, they tend to miss the objective and select for total performance of the varieties while evaluating the experiment. In case of sugarcane, the criterion changes that increase in sugar per unit area is measured. If results were found statistically insignificant, the trials were abandoned. The same situation exists with mutation breeding and varieties obtained through tissue culture. On many occasions, what may be statistically insignificant could be very significant on a national level for a specific character, e.g. increase of sucrose concentration in juice for a variety may only be to 0.4 ton per hectare and statistically insignificant in that particular trial. When calculated on a national level this could give rise to very significant increases. Therefore, the testing and selection should be one which incorporates safeguards and yield results to the situation. The Fijan soils are generally poor and highly deficient in organic matter, phosphorus and potassium. Thus, a plant breeder has to produce varieties which have all the required attributes (high yield of sugar per unit area and good milling qualities such as, uniform fiber i.e. 10.5 to 13.5% with a seasonal average of 12.5%; about 7.2 - 7.8 tons cane to make a ton of sugar, low impurity loading, etc.). These are substantial demands on any breeding program especially where the worst diseases and pests of cane such as Fiji disease, downy mildew (*Sclerospora sacchari* Miy). and nematodes are present.

With the above factors in view, our breeding and early selection program was modified to fulfill the needs in a manner that it will produce varieties with good field attributes high resistance to pests and diseases, and improved milling qualities.

MATERIALS AND METHODS

In any one year, we effected about 1,200 crosses. The seed for planting was drawn from selected 800 crosses with a maximum of 400 seedlings from each cross. In the new system, we usually have a population of 20,000 seedlings planted in the field in intensive care trial (ICT) as will be described later. The crosses selected in the first stage were from the following categories:

Commercial x commercial e.g. Homer x Mali
Commercial and near commercial polycrosses (Melting pot crosses)
Interspecific crosses e.g. *S. officinarum* x *S. spontaneum:*
S. officinarum x *S. robustum*
Intergeneric crosses e.g. *S. officinarum* x *Brianthus.*
S. officinarum x *Riphidium*

Backcrosses

The 20,000 seedlings were planted in ICT in the field. In the ICT, the seedlings were planted at 30 cm spacing in rows which were 75 cm apart giving a population density of 99,000 stalks per ha. The canes when grown were limited to two stalks and supported by wire frames. Five known commercial varieties were planted as standards. Each standard made up a minimum of one percent of the total population. Thus, the standards always constitute five percent of the total seedling population. The selected population from seedlings i.e. stage 2 and the subsequent selections stage 3 were planted in pots (Daniels and Stevenson[3]) with three replications each. The same varieties were also planted in 2 m plots in the field so that all fields characters can be assessed under appropriate conditions. The field characters recorded were: flowering stalk length and thickness, tillering, stooling, side shooting, suckering, trashing, lodging, diseases and general agronomic desirability.

The basis of selection for all population for seedlings, stages 2 and 3 were on ratings arrived at after calculating for the standards against their known ratings. The ratings 0 to 9 were used (Daniels and Horsely[5]); 0 indicated the best of performance and 9, the worst. The discard level was placed a unit below the least performing standard. The known ratings for Fijian standard varieties were as follows:

	SUC	FIB	TCH	TSA	FIJ	DOM
Ragnar	2	3	4	3	2	2
Homer	2	2	6	6	2	9
Waya	6	4	2	2	2	7
Mali	6	6	3	3	4	1
Vomo	4	6	6	5	2	2
Spartan	2	2	3	3	2	6
Yasawa	2	2	4	5	1	3
Mana	4	2	4	5	1	6
LF51-124	2	7	2	2	4	5

DOM = Downy mildew
FIJ = Fiji disease
TSA = Tons sucrose/ha
TCH = Tons cane/ha
FIB = Fiber percent
SUC = Sucrose-Brix Fresh wt.

Once the standards were brixed, and the fiber measured and cane weighed, the regression was calculated and the regression line was graphed (Fig. 1).

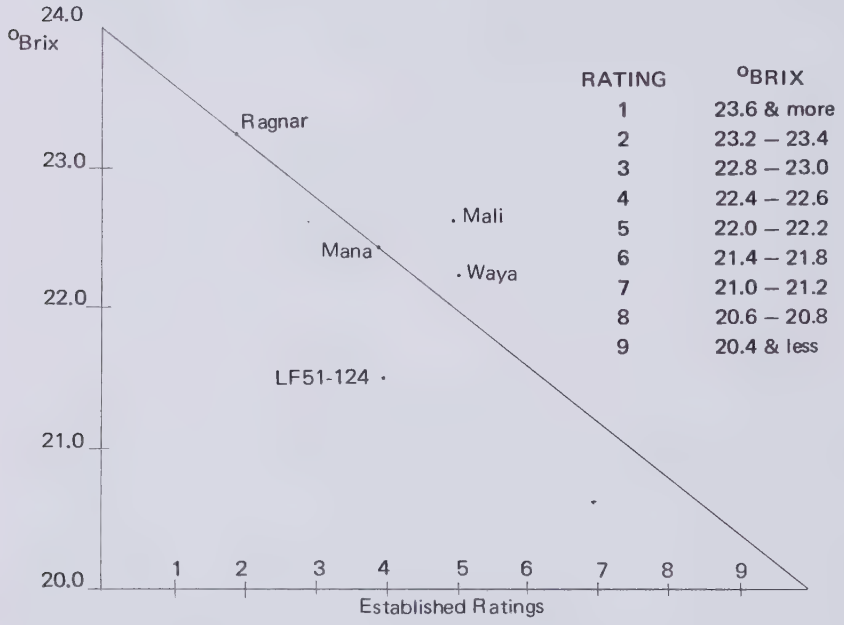

FIGURE 1. Regression Line Graph for Sucrose Selection

When the different characters were measured for test varieties, the ratings were assigned using the system in Table 1. The data was then entered into computer sheets for storage and analysis. The sample of the sheet is attached as Appendix 1.

TABLE 1. System of rating used when measuring varietal characters.

	Actual		Known rating		Given rating	
	Brix	Fiber	Brix	Fiber	Brix	Fiber
Ragnar	23.4	11	2	2	1 = 23.1 - 16+	9
Mana	22.6	10	4	2	2 = 23.2 - 23.4	10
Mali	22.6	14	6	6	3 = 22.8 - 23.0	11
Waya	22.4	12	6	4	4 = 22.4 - 22.6	12
LF51-124	21.6	15	2	7	5 = 22.0 - 22.2	13
					6 = 21.4 - 21.8	14
					7 = 21.0 - 21.2	15
					8 = 20.6 - 20.8	16
					9 = 20.4 - Less	17 plus

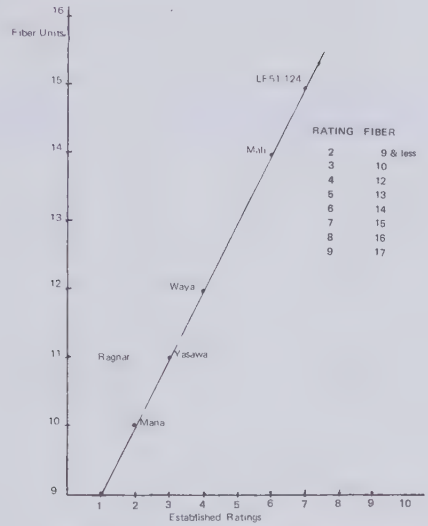

FIGURE 2. Regression Line Graph for Fiber Selection

The selections at various stages were made on the following criteria:

Stage of Selection	Population	Characters
1	20,000	SUC, FIB.
2	5,000 x 3 reps	SUC, FIB, TCH.
3	3,000 x 3 reps	SUC, FIB, TCH, RS. TS. All field characters and diseases DOM & FIJ.
4	600	Seed Multiplication
5	2 (reps) 2 x 600 x 12 locations Mass stool population	TS, RS, SUC, FIB, TCH, TSH, STARCH, All field characters.
6	Randomized block x 6 reps. 4 row x 20 meters	All field and bio-chemical characters.
7	Large plots	Large mill test.

The stage five selection was based on mass stool population principles (MSP) (Daniels J. *et al*[4]). From the original concept of MSP, the following variations have been introduced:

* The entire stool was allowed to grow, i.e. free stooling. This was used for measuring yield.
* Although ratoon data was considered for all selection purposes we do not wait for ratoon data for initial selections. This was incorporated later.
* The trials were in two replications in twelve locations.

RESULTS

All the selections were based on measurable characters in comparison with the standards. The selections made to date were shown in Table 2.

TABLE 2. Selections made from 1971 to 1978 on Stages 1 to 5.

Stages	1971	1972	1973	1974	1975	1976	1977	1978
1	12,248	10,000	3,060	17,100	28,516	22,090	22,164	20,029
2	4,700	5,107	1,560	4,473	9,915	5,284	5,138	
3	4,700	3,000	1,175	2,300	3,259	1,982		
4	1,636	1,874	1,175	1,389	1,839			
5	278	428	386					

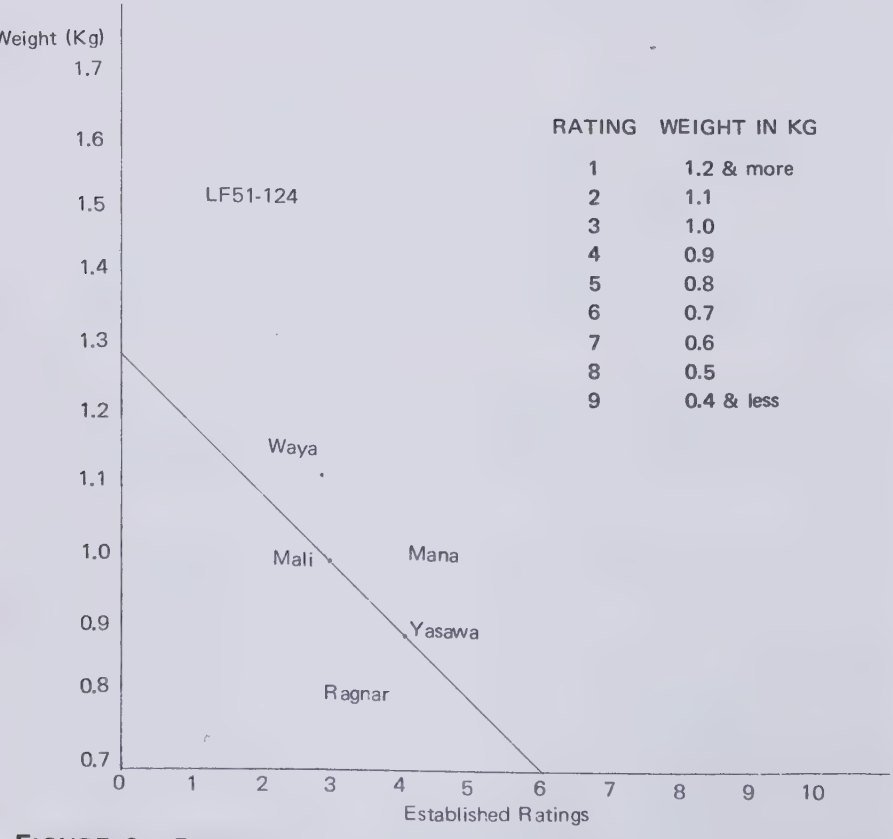

FIGURE 3. Regression Line Graph for Yield Selection

DISCUSSION

The current system eliminated human errors caused by the operator as all selections were based on measurable characters, thus minimizing personal bias. The system also eliminated much of the error coming from the environment. Row spacing, soil type, moisture, competition for light, etc. were all made uniform. The interaction to the environment was well tested at stages five and six by having the stage 5 MSP trials at twelve locations in two replications each, and stage six trials in at least four different sites. The interaction assessed for were: soil type; maturity as affected by soil, rainfall, and night temperatures; infection by sugarcane borers and nematodes; and year to year variations on the same location. In essence the interactions were measured on two parameters: within the year over various locations, and on the same location over the years.

Such a system, therefore, offered a plant breeder greater latitude of operation than previously possible. However, it must be observed that the system required the facilities of a computer and its operators to measure the various characteristics of large populations.

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ETAPAS TEMPRANAS DEL PROGRAMA DE SELECCION DE VARIEDADES EN FIJI

M. Krishnamurthi y S. C. Prasad

RESUMEN

Se ha diseñado un nuevo esquema para la selección de variedades de caña de azúcar en las etapas tempranas del programa.

APPENDIX 1. Computer sheet for storage and analysis.

C A N E D A T A **CANDAF** CANE DATA FILE

MILL: DATE:

NAME: PAGE OF

TRIAL OR REF	YEAR	MONTH	CLONE OR CROSS	No. PLTD	No. SCTD	CODED DATA: AGD	BREED: FLT	SEX	FEP	PCF	AGRICULTURAL: STAF	TRAL	TILE	LSUK	SSHE	STLER	RSUC	FSIB	SCCE	SCCM	TCLA	TSAA	PATH: FIJ	DOM	LSC	YES	RTG	CHC	NON-CODED DATA FROM MATURITY AND SELECTION TRIALS: SRG	SYS	JUN	JLY	AUG	SEP	OCT	NOV	DEC
1 2 3 4 5 6	7	8	9 10 11 12 13 14 15 16 17 18 19 20	21 22 23 24	25 26 27	28	29 30 31 32	33	34 35 36 37 38 39 40 41 42 43 44 45 46 47	48	49 50 51 52	53	54 55 56	57 58	59	60 61 62	63 64 65	66 67 68	69 70 71	72 73 74	75 76 77	78 79 80															

Esta esquema se basa en la selección de caracteristicas individuales tal como los requerimientos industriales los proponen. En el procedimiento anterior por lo contrario, tenia mayor valor el comportamiento total.

CORRELATIONS AMONG CHARACTERS OF SUGARCANE IN TWO INTERMEDIATE SELECTION STAGES

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ABSTRACT

Correlation coefficients among seven agronomic characters of sugarcane were studied from data collected on 93 clones grown in the plant crop of Stage II at one location and the same clones grown in the plant and first ratoon crops of Stage III at 4 locations. Results indicated that stalk number was negatively correlated with stalk weight, but both of these characters were positively correlated with tons per hectare of cane and tons per hectare of sugar. A compromise must therefore be made between the optimum stalk number and stalk weight. The multiple correlation of Brix and sucrose percentage with sugar per ton of cane was as large as their simple correlations. The combined effect of Brix and sucrose on either tons per hectare of cane or tons per hectare of sugar was moderate. Stalk number, stalk weight, Brix, sucrose percentage, and sugar per ton of cane were highly repeatable between crops. Two characters, ton per hectare of cane and ton per hectare of sugar, were not repeatable between selection stages. Predictability of Stage III performance on the basis of Stage II plant crop performance was evaluated by regression coefficients. Neither ton per hectare of cane nor ton per hectare of sugar from the plant crop of Stage II was an effective predictor of performance in the plant and first ratoon crops of Stage III.

INTRODUCTION

The literature on correlations among various characters in sugarcane is vast (Coleman *et al*[3], Hebert and Henderson[5], James and Falgout[8], Loupe *et al*[13], Miller and James[17], and Smith and James[18]), but relatively few investigations have involved correlations between two selection stages. Smith and James[18] reported that phenotypic correlations among number of stalks, diameter of stalks, and Brix were relatively low within plant cane and first ratoon crops of progenies of four sugarcane crosses. The repeatability of these characters between crops was highly significant. Among major components of yield and quality, stalk number was found to have the highest degree of repeatability by Hebert and Henderson[5], James and Miller[10], Brown *et al*[2], and Ladd *et al*[11], whereas juice quality was reported to be most repeatable by Hogarth[6] and Mariotti[14]. Miller and James[17] also reported that repeatability for stalk diameter among plant crops was somewhat higher than that for stalk number and Brix, which were about equal. Repeatability for stalk number and diameter was about equal between plant crop and first ratoon crop, but Brix was more repeatable among ratoon than among plant crops. Allam *et al*[1] reported that in Louisiana, the predictability of outfield performance from results of infield tests was very poor. Mariotti[15] measured the effectiveness of clonal selections in sugarcane and found that the diameter of stalks and weight per stalk appeared to be the most stable traits with respect to their expression in different localities.

The USDA sugarcane selection scheme at Canal Point consists of seedlings, Stages I, II, III and IV (Falgout *et al*[4] and Miller[16]). In recent years about 12 to 15% of the clones from Stage II have been advanced to Stage III, which were replicated trials at four locations. The objective of this study was to investigate correlations of certain agronomic characters of sugarcane clones within Stages II and III and the repeatability for a given character between those two selection stages. These data should permit us to improve the selection effectiveness and efficiency in our sugarcane varietal development program.

MATERIALS AND METHODS

Ninety-two sugarcane clones and the check variety CP 63-588 were used for analysis. Stage II clones were planted in 1974 in single two-row plot (4.57 m X 3.05 m) on the Canal Point Station. Millable stalks per plot were counted when the cane was 10 months old. A sample of 10 millable stalks from cane about 12 months old was taken from each selection and milled. Stalk weight, Brix, and sucrose percentage were measured on each sample. Estimates of sugar per ton of cane, and of cane and sugar yield per hectare were obtained by multiplying by the appropriate factors. All 93 clones were planted in 1975 at each of the four Stage III trials located in the major cane-growing areas. These locations were Hatton Bros. Farm at Pahokee, University of Florida Agricultural Research and Educational Center at Belle Glade, Shawnee Farm at Clewiston, and Gulf & Western Food Products Co. at Okeelanta. Each clone was planted in two replications in two-row plots (3.05 m x 4.57 m). Characters measured in all plots of plant and first ratoon crops of Stage III were the same as those measured in Stage. II.

All possible simple correlations were calculated between the seven characters measured. Multiple correlation coefficients (Little and Hills[12], Steel and Torrie[19]) were also calculated to study the combined effect of the independent variables (stalk number, stalk weight, Brix, and sucrose percentage) on the dependent variables (sugar per ton of cane, ton per hectare of cane, and ton per hectare of sugar). Correlations for a given character between stages and between crops were considered to be estimates of repeatability (Miller and James[17], Smith and James[18]). Regression analyses were conducted to determine the predictability of Stage III performance from Stage II performance (Allam *et al*[1], Little and Hills[12], Steel and Torrie[19]).

RESULTS AND DISCUSSION

Correlations

The results from plant crops of stages II and III (Table 1) indicated that neither stalk number nor stalk weight was significantly associated with Brix, sucrose percentage, or sugar per ton of cane. In the first ratoon crop of Stage III neither stalk number nor stalk weight was closely associated with Brix or sugar per ton of cane. The associations were relatively low and probably would not be of great importance in selecting clones with desirable combinations of characters. These data agreed with those obtained by Smith and James[18] from unselected populations.

Data from plant crops of Stages II and III indicated that stalk number was negatively correlated with stalk weight, but both characters were positively associated with tons per hectare of cane and tons per hectare of sugar. Correlation coefficients for these characters in the first ratoon crop of Stage III were similar to those in the plant crop. Stalk number and Brix were not correlated in either stage, a result that contrasted with that reported by Smith and James[18]. Stalk number had a higher correlation than stalk weight to tons per hectare of cane and tons per hectare of sugar in both plant and first ratoon crops of Stage III. Although a compromise in both stalk number and stalk weight may be desirable to increase the frequency of high-yielding clones in selected populations (James[7]), more emphasis should be placed on stalk number in later selection stages to increase the probability of developing high-yielding varieties with better stubbling ability. The correlation coefficient between stalk number and tons per hectare of cane was 0.51 for the plant crop of Stage II, which was close to the 0.58 correlation for the selected population of plant cane reported by James[7]. However, in this study the population in Stage III had higher correlation coefficients between those two characters in both plant (pooled $r = 0.63$) and first ratoon (pooled $r = 0.81$) crops than it did in Stage II.

Brix and sucrose were positively correlated in plant crops of both Stages II and III. Both of these two characters were highly positively associated with sugar per ton of cane, moderately associated with tons per hectare of sugar and poorly associated with tons per hectare of cane. Correlations in the ratoon crop of Stage III were similar to those in the plant crop of the same stage. The low correlation

TABLE 1. Correlation coefficients between the seven characters of 93 clones of sugarcane from two stages of the USDA sugarcane selection program.*

Correlation [2]	Stage II	Stage III Plant Crop Location [1]					Stage III Ratoon Location [1]				
	Canal Point	BG	HAT	SHA	OKE	Pooled	BG	HAT	SHA	OKE	Pooled
SN-SW	-0.41	-0.55	-0.53	-0.42	-0;46	-0.60	-0.28	-.047	-0.22	-0.42	-0.46
SN-B	-0.02	-0.20	-0.08	-0.00	0.05	-0.08	0.01	0.07	-0.01	0.15	00.05
SN-S	-0.11	-0.21	-0.01	0.06	0.04	-0.06	-0.05	0.08	0.05	0.13	0.04
SN-S/T	-0.13	-0.21	0.02	0.08	0.04	-0.05	-0.07	0.08	0.07	0.12	-.03
SN-THC	0.51	0.63	0.54	0.78	0.66	0.63	0.80	0.70	0.84	0.83	0.81
SN-THS	0.44	0.45	0.49	0.73	0.60	0.56	0.72	0.70	0.83	0.79	0.80
SW-B	-0.20	-0.10	-0.02	0.04	0.01	-0.06	-0.15	-0.22	-0.04	-0.09	-0.18
SW-S	-0.19	-0.13	-0.15	-0 05	-0.03	-0.13	-0.16	-0.25	0.09	-0.11	-0.22
SW-S/T	-0.18	-0.14	-0.18	-0.08	-0.04	-0.15	-0.16	-0.26	-0.11	-o.11	0.24
SW-THC	0.56	0.28	0.40	0.22	0.35	0.23	0.35	0.27	0.32	0.14	0.12
SW-THS	0.44	0.17	0.22	0.18	0.30	0.11	0.25	0.12	0.26	0.08	0.01
B-S	0.93	0.95	0.84	0.93	0.96	0.96	0.97	0.96	0.95	0.98	0.97
B-S/T	0.88	0.91	0.73	0.87	0.93	0.93	0.95	0.90	0.90	0.96	0.95
B-THC	-0.19	-0.32	-0.16	0.04	0.06	-0.18	-0.10	-0.12	-0.06	0.12	-0.08
B-THS	0.31	0.40	0.33	0.40	0.47	0.40	0.38	0.35	0.21	0.46	0.34
S-S/T	0.99	0.99	0.98	0.99	1.00	1.00	1.00	0.99	0.99	1.00	1.00
S-THX	-0.27	-0.36	-0.16	0.04	0.01	-0;21	-0.16	-0.13	-0.02	0.08	-0.12
S-THS	0.29	0.42	0.49	0.43	0.45	0.42	0.35	0.37	0.27	0.44	0.32
S/T-THC	-0.29	-0.36	0.04	0.00	-0.21	0.18	-0.14	-0.01	0.06	-0.13	-0.15
S/T-THS	0.27	0.42	0.51	0.43	0.44	0.42	0.34	0.37	0.43	0.43	0.31
THC-THS	0.84	0.68	0.76	0.91	0.89	0.79	0.86	0.86	0.95	0.95	0.90

* $r \geq 0.204$ ($P = 0.05$); $r \geq 0.266$ ($P = 0.01$).

1 BG = Belle Glade; HAT = Hatton Bros. Farms; SHA = Shawnee Farm; OKE = Okeelanta.

2 SN, stalk number: SW, stalk weight; B, Brix; S, sucrose; S/T, Kg of sugar per ton of cane; TCH, tons per hectare of cane; THS, tons per hectare of sugar.

between sucrose and tons per hectare of cane should permit selection of high-sucrose varieties with high values for tons per hectare of cane.

Correlation coefficients between stalk number and tons per hectare of cane, between stalk number and tons per hectare of sugar, and between tons per hectare of cane and tons per hectare of sugar were significant in the plant and first ratoon crops of Stage III, but were higher in the ratoon crop than in the plant crop.

The correlation coefficients reported in Table 1 were about the same as those reported by Smith and James[18] for most characters. Correlation coefficients from the plant crops of Stages II and III and first ratoon crop of Stage III indicated that stalk weight was as reliable in performance as stalk diameter as reported by James and Miller[9,10].

TABLE 2. Multiple correlation coefficients between each of the dependent variables (S/T, THC and THS) and the independent variables (SN, SW, B, S) taken two at a time, as measured with 93 clones from two stages of USDA sugarcane selection program*.

Partial correlation[1]	Stage II plant crop	Stage III plant crop	Stage III first ratoon crop
$R_{S/T.SN, SW}$	0.28	0.28	0.25
$R_{S/T.SN,B}$	0.90	0.91	0.95
$R_{S/T.SN,S}$	0.91	1.00	1.00
$R_{S/T.SWB}$	0.88	0.93	0.91
$R_{S/T.SW,B}$	0.99	1.00	1.00
$R_{S/T.B,S}$	0.99	1.00	0.91
$R_{THC.SN,SW}$	0.98	0.93	0.97
$R_{THC,SN,B}$	0.54	0.70	0.81
$R_{THC,SN,S}$	0.55	0.65	0.82
$R_{THC.SW,B}$	0.56	0.28	0.34
$R_{THC.SW,S}$	0.57	0.28	0.25
$R_{THC,B,S}$	0.32	0.22	0.59
$R_{THS.SN,SW}$	0.81	0.79	0.90
$R_{THS.SN,B}$	0.54	0.71	0.81
$R_{THS.SN,S}$	0.56	0.70	0.84
$R_{THS.SW,B}$	0.59	0.42	0.34
$R_{THS.SW,S}$	0.58	0.42	0.33
$R_{THS.B,S.}$	0.36	0.45	0.33

** $R = 0.254$ ($P = 0.05$); $R = 0.312$ ($P = 0.01$).*

1 $R_{S/T.SN,SW}$ = Multiple correlation coefficient for combined affect of the independent variables on the dependent variable, SN, stalk number; SW, stalk weight; B, Brix; S, sucrose percentages; S/T, sugar per ton of cane; THC, tons per hectare of cane; THS, tons per hectare of sugar.

Multiple Correlations

Multiple correlations that measure the combined relation between one character and two other characters varying simultaneously are presented in Table 2. They were calculated on a basis of pooled data from 4 locations. The simple correlation of either stalk number or stalk weight alone with sugar per ton of cane were negative in most cases and not very large at either Stages II or III (Table 1). When those two characters were considered simultaneously, the relation wtih sugar per ton of cane was significant but still not very close (Table 2). The combined effect of stalk number and stalk weight on both tons per hectare of cane and tons per hectare of sugar was very large. The multiple correlation of sugar per ton of cane with Brix and sucrose was as close as their simple correlations. However, the combined effect of Brix and sucrose on either tons per hectare of cane or tons per hectare of sugar was not very large.

Repeatability

Individual repeatability was estimated by the correlations between stages and between crops for a given character (Table 3). Of the seven agronomic characters examined, five had relatively high correlation coefficients between plant crops of Stages II and III and plant crop of Stage II and first ratoon crop of Stage III. Thus, stalk number, stalk weight, Brix, sucrose percentage, and sugar per ton of cane had a relatively high degree of repeatability between crops. Two characters, tons per hectare of cane and tons per hectare of sugar had low associations between selection stages. These associations suggested that both tons per hectare of cane and tons per hectare of sugar were not very repeatable based on plant crop performance of Stage II. Allam *et al*[1] also reported that tons per hectare of cane and tons per hectare of sugar from in field selection stage were not significantly correlated from the same data from outfield plant crop. A stage-to-stage repeatability of 0.42 (from the pooled value between plant crops of Stages II and III) and 0.45 (from the pooled value between the plant crop of Stage II and the first ratoon crop of Stage III) for stalk number were very close to the value of 0.39 as reported by Mariotti[15]. Correlation coefficients between crops of Stage III were 0.82 and 0.84 for stalk number and Brix, respectively (Table 3). Based on unselected populations, Smith and James[18] obtained coefficients of 0.611 and 0.389 for correlations of these two characters between plant and first ratoon crops, respectively. Thus, the selected populations had higher degrees of repeatability for these characters between crops than did the unselected ones.

Predictability

Predictability of Stage III performance on the basis of Stage II plant crop performance was evaluated by regression coefficients as shown in Table 4. For tons per hectare of cane and tons per hectare of sugar, the regression of both plant and first ratoon crops for Stage III on the performance of the plant crop of Stage II was low and non-significant. Thus, neither tons per hectare of cane nor tons per hectare of sugar in the plant crop of Stage II effectively predicted the performance in Stage III. Allam *et al.*[1] also reported that for tons per hectare of cane and tons per hectare of sugar, the regression of outfield plant crop on infield plant crop was non-

TABLE 3. Correlation coefficients for seven characters of 93 clones of sugarcane from the USDA selection program, correlated between different stages and between crops*.

Correlation	Location[2]	Agronomic Character[1] SN	SW	B	S	S/T	THC	THS
Stage III Plant Crop vs Stage II Plant Crop	BG	0.42*	0.67	0.56	0.59	0.58	0.09	0.01
	HAT	0.41	0.56	0.40	0.38	0.36	0.17	0.06
	SHA	0.30	0.66	0.45	0.55	0.57	0.06	0.03
	OKE	0.42	0.56	0.61	0.65	0.64	0.05	0.04
	Pooled	0.42	0.67	0.58	0.62	0.62	0.11	0.04
Stage II First Ratoon Crop vs stage II Plant Crop	BG	0.44	0.60	0.53	0.53	0.53	0.08	-0.02
	HAT	0.41	0.56	0.43	0.42	0.41	0.10	0.02
	SHA	0.31	0.56	0.52	0.48	0.45	0.05	-0.08
	OKE	0.48	0.58	0.54	0.59	0.59	0.02	0.02
	Pooled	0.45	0.65	0.58	0.58	0.57	0.08	-0.03
Stage III First Ratoon Crop vs Stage III Plant Crop	BG	0.77	0.78	0.73	0.72	0.71	0.66	0.56
	HAT	0.77	0.63	0.70	0.77	0.73	0.44	0.43
	SHA	0.75	0.70	0.62	0.66	0.65	0.61	0.51
	OKE	0.76	0.76	0.84	0.81	0.80	0.60	0.59
	Pooled	0.82	0.88	0.84	0.84	0.83	0.70	0.62

**R = 0.254 (P = 0.05); R = 0.0266 (P = 0.01)*

[1]SN, stalk number; SW, stalk weight; B, brix; S, sucrose; S/T, sugar per ton of cane; THC, tons per hectare of cane; THS, tons per hectare of sugar.

[2]BG, Belle Glade; HAT, Hatton Bros.; SHA, Shawnee; OKE, Okeelanta.

TABLE 4. Regression of Stage III plant crop on Stage II plant crop, Stage III first ratoon crop on Stage II plant crop, and Stage III first ratoon crop on Stage III plant crop for seven agronomic characters of 93 sugarcane clones from the USDA selection program.

Regression	Location[2]	Agronomic character[1] SN	SW	B	S	S/T	THC	THS
Stage III plant crop on Stage II plant crop	BG	0.386	0.602	0.631	0.627	0.605	0.096NS	0.001NS
	HAT	0.343	0.534	0.422	0.338	0.312	0.167NS	0.042NS
	SHA	0.304	0.597	0.439	0.446	0.439	0.073NS	0.041NS
	OKE	0.358	0.464	0.598	0.588	0.570	0.042NS	0.048NS
	Pooled	0.348	0.549	0.522	0.500	0.482	0.095NS	0.038NS
Stage III first ratoon crop on Stage II plant crop	BG	0.428	0.356	0.511	0.493	0.474	0.080NS	-0.042NS
	HAT	0.418	0.345	0.392	0.305	0.274	0.085NS	-0.014NS
	SHA	0.392	0.374	0.580	0.406	0.345	0.064NS	-0.155NS
	OKE	0.504	0.340	0.655	0.574	0.528	0.014NS	0.005NS
	Pooled	0.436	0.354	0.534	0.444	0.405	0.061NS	-0.051NS
Stage III first ratoon crop on Stage III plant crop	BG	0.811	0.520	0.640	0.648	0.634	0.612	0.549
	HAT	0.934	0.413	0.599	0.652	0.586	0.397	0.339
	SHA	0.936	0.565	0.714	0.738	0.704	0.640	0.558
	OKE	0.961	0.534	1.026	0.855	0.789	0.593	0.569
	Pooled	0.985	0.583	0.876	0.820	0.786	0.695	0.612

NS Not significant at the 5% level of probability. The other unmarked values indicate significance at the 1% level of probability.

1 SN, stalk number; SW, stalk weight; B, Brix; S, sucrose percentage; S/T, sugar per ton of cane; THC, tons per hectare of cane; THS, ton per hectare of sugar.

2 BG, Belle Glade; HAT, Hatton Bros. Farm; SHA, Shawnee Farm; OKE, Okeelanta.

significant. They also reported that infield and outfield performance for sugar per ton of cane were not consistently correlated. However, this study indicated that regression of the performance of both Stage III crops on the performance of the Stage II crop for sugar per ton of cane was significant, and that sugar per ton of cane would serve as a reliable selection criterion.

An increase of one unit of Brix in plant cane of Stage II was significantly associated with an average increase of 0.522 unit in the plant crop of Stage III and of 0.534 unit in the first ratoon crop of Stage III. For stalk number, stalk weight, sucrose percentage, and sugar per ton of cane, the regression of Stage III on Stage II was significant. The plant crop performance of Stage III gave very high predictability for the first ratoon crop performance of the same stage in all seven characters examined.

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CORRELACIONES ENTRE CARACTERES DE CAÑA DE AZUCAR EN DOS ESTADIOS INTERMEDIOS DE SELECCION

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RESUMEN

Se estimaron los coeficientes de correlación para 7 características agronómicas de la caña de azúcar, sobre la base de datos tomados en 93 clones en el estadio de selección II (caña planta) y en el estadio III (caña planta y primera soca), éste último en 4 localidades.

Los resultados indican que el número de tallos está negativa-

mente asociado al peso de los tallos, a pesar de que ambos presentan una asociación positiva con el rendimiento de caña y de azúcar por hectarea. Debe por tanto, buscarse un equilibrio entre estas dos caracteristicas. La correlación múltiple entre brix, porciento de sacarosa y azúcar por tonelada de caña fué tan elevada como las correlaciones simples correspondientes. El efecto combinado del brix y del porciento de sacarosa fué moderado en relación al rendimiento de caña o de azúcar por hectarea. Se observó una alta repetibilidad para caracteristicas tales como número de tallos, peso de tallos, brix, porciento de sacarosa y azúcar por tonelada de caña. En cambio toneladas de caña o de azúcar por hectarea no mostraron repetibilidad entre etapas de selección. La capacidad de la etapa II para predecir el comportamiento en la etapa III fué evaluada por estudios de regresion. Ni las toneladas de caña ni las de azúcar por hectarea en la etapa II fueron pronósticos efectivos del comportamiento en planta o soca en la etapa III.

EXPERIMENTAL METHODOLOGY FOR FIELD RESEARCH WITH VARIETIES OF SUGARCANE IN CUBA

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ABSTRACT

Different factors affecting the accuracy and reliability of field experiments with sugarcane such as size and shape of experimental plots, optimum number of replication, effect of inner borders, size and optimum number of samples for sugar analysis and crop estimation at experimental areas in place of weighing were analyzed.

No significant differences were observed in plots having from 3 to 6 furrows. In the analyzed varieties, inner border effect was not evident. In applying a random sampling per particular stalks, four samples of five stalks should be taken in each plot. The crop estimating method can replace the crop weighing one.

INTRODUCTION

Various factors affect the accuracy of field experiments, notable of which were the same and size of experimental plots, number of replications used in experimental designs, possible effect of inner and outer borders, and the size of optimum number of samples for sugar analysis.

Previous studies (Pearse[15], Iyer and Agrawal[8]) had shown that when the shape of plots increases in length and decreases in width, the smaller values of the error unit variance were obtained. However, when the blocks were taken into account, the compact shape provided better results than the former. On the other hand, Abreu[1] and Iyer and Agrawal[8] obtained optimum plots of 40 and 48 m^2 respectively.

As to the possible effect of the borders, Heri[6] stated that the only significant effect was that of taller over shorter varieties. Fuentes[5] and Loves[10] considered that while trying to cut our border effect, relevant errors such as the ones intended to be avoided can be brought about. Lalouette[9] expresses that the work involved in this operation would be twice if border effect is not taken into account.

Herr[7], Arcenaux[3] and Olivera[14] stated the advantages of random sampling per particular stalks for determining the optimum size of samples in sugar analysis of the cane. Skinner [16], Diaz[4] and Alvarez and Ortiz[2] on the other hand worked on the estimation of cane weight in experimental plots. Milanes [11,12,13] found

out there was no significant effect of plots area on the size and number of the samples, as long as such area was not over 100 m^2, both in the plant and in the first sprout, and that there were insignificant differences among the sizes of the analyzed samples. Furthermore, the number of samples to be taken in each experimental plot should be no less than 4 samples and that the best method to estimate the cane yield in experimental plots is that in which two furrows—one inner and one outer — are considered and a sample consisting of 20 stalks of the whole area are cropped. Milanes [11,12,13] also recommended the formula to estimate cane in the plots of four cropped furrows:

$$P_p = \frac{T_c \times P_m}{K} \qquad (1)$$

where: P_p = weight of the plot, expressed in kg

T_c = stalks reckoned in the 2 furrows

P_m = weight of the sample with 20 stems

K = constant with a value of 10

He concluded that some major aspects on experimental methods for sugar cane field studies were still to be discussed.

In this light, the present study was conducted in the Sugarcane Experimental Station, at the "Hector Molina" sugar mill of Havana, on red ferralitic soil to determine the area and shape of the experimental plots and optimum number of replications for sugarcane experiments, study inner border effect and determine the size of samples for sugar analysis per particular stalks and the variability among and within the stools.

MATERIALS AND METHODS

1. Determining the area and shape of the experimental plots and optimum number of replications for sugarcane experiments.

The area cropped comprised 4800 m^2. The analyzed variety was C 87-51 for two types of stubs (cane plant and first ratoon). A 4 x 2 x 10 factorial design was used.

Four blocks were arranged in continuous furrows with a 1.60 m ridge where each block represented plot, i.e. F_1, F_2, F_3 and F_4 fitted 3,4,5, and 6 furrows to be cropped, respectively. Each block consissted of 10 plots of 120 m^2. These plots were subdivided in unit plots of 10 m^2, each, totalling 120 plots for each block. With these base areas 15 combinations were obtained (Table 1).

The studied parameter was cane kg/plots and the typical error was applied as starting point for the statistical evaluation of data; the latter was appraised in a factorial variance analysis, considering the variation involved due to the types of stubs, shape and area of the plots. The areas that provided better results were ascertained of the optimum number of replications and in each separate area a

TABLE 1. Combinations of sizes of areas and number of studied replications.

Area	Number of plots	Replications
I. 10 m^2	1	102
II. 20 m^2	2	60
III. 30 m^2	3	40
IV. 40 m^2	4	30
V. 50 m^2	5	24
VI. 60 m^2	6	20
VII. 80 m^2	8	15
XI. 100 m^2	10	12
X. 120 m^2	12	10
XI. 150.m^2	15	8
XI. 200 m^2	20	6
XII. 300 m^2	30	4
XIII. 400 m^2	40	3
XIV. 500 m^2	50	2
XV. 600 m^2	60	2

variance analysis of two fold classification on standard errors was carried out.

2. *Inner border effect in comparative experiments of varieties*

Varieties B 4362, C 87-51 and Ja 60-5 were selected to appraise the effect of inner borders in cane plant and they were sown in a random block design with 4 replications. The plots had 6 furrows, 7.5 m long each.

The evaluation of the first ratoon was based on a comparative analysis of 12 varieties: C 334-64, C 227-59, C 116-63, C 100-59, My 6426, My 6414, My 6410, Ja 64-19, Ja 64-11 and the aforementioned three. They were sown in a random block design with 4 replications with each plot consisting 6 furrows. In all cases the outer border of the experimental field were maintained.

Harvesting was done on single furrows in each plot, with the following parameters, used for the analysis of cane plant: cane in kg/furrow, brix in %, % of Pol in juice, number of stalks when it is 6 months old and number of stalks at the age of 12 months. In the study of varieties in first ratoon the only parameter that was appraised was cane in kg/furrow. For sugar analysis 5 stalks/furrow were taken.

The statistical evaluation was carried out by means of variance analysis of twofold classification. The F and Duncan tests were applied at 5% level of probability. The furrows were analyzed, separately and then in groups composed of outer, middle and inner ones.

3. *Determining the size of samples for sugar analysis per single stalks, variability among and within the stools and checking of the derived estimating method.*

Varieties analyzed

Varieties C 87-51, C 353-69 and C 568-69 were used in sampling for sugar analysis. To check the final method of estimation, seventeen were studied. Some of them, such as the B 4362, C 87-51, Ja 60-5 and My 54129 are widely spread in the cane fields of the country.

Samplings

The crop duration was divided in three stages of 45 days each: a) From December 1st to January 15, b) From January 16 to February 29 and c) From March 1st to April 15 to fulfill the samplings during well-distinguished points of time in the process of ripening and harvesting of sugarcane. Observations were made on cane plant and first ratoon.

Analysis were made of Brix in %, weight/stalks in kgs and % of Pol in juice, besides stalk weighing and reckoning in the estimation study. All the studies were conducted under an experimental design of one, two or three factors, in random blocks as well as in factorial. They were evaluated through the corresponding variange, applying subsequently the F and Duncan test at a 1 and 5% levels of probability.

A plot of 16 furrows, 12 m long, was sown for each variety (C 353-69, C 87-51 and C 568-69) continuously, to determine the size of the samples for sugar analysis. A systematic sampling per single stalks was applied on these plots, in the 12 central furrows, with each stalk taken at 0.8 m equidistantly. In each variety sampling 144 stalks (12 x 12) were obtained, the weight in kg, Brix in % and % of Pol in juice were determined in each stalk and two samplings were carried out in cane plant and two others in first ratoon. A variance analysis was used on primary data and if there were no relevant differences between rows and columns, only those combinations which proved better were taken out of the 144 stalks. In this way, the 32 combinations of sample sizes were obtained (Table 2).

Varieties C 87-51, C 353-69 and C 568-69 were analyzed to determine the variability among and within the stools. With each variety a plot of 10 furrows, 12 m long, was sown; the only sampling of cane plant was carried out on March 24, 1976 and on first ratoon 3 samplings were put into practice. The first was on December 15, 1976; the second on February 2, 1977 and the third on March 15, 1977. In each of the plots, according to variety and sampling 10 stools are taken at random and 5 milled stalks were taken from the outer part towards the center of the stool, selecting stalks from all layers of the tillering polynomial. These stalks were strictly specified according to their position in the stool and a laboratory analysis was performed on each to determine the % of Brix and the % of Pol in juice. All the information were analyzed by means of a variance analysis of two factors (within and among stools) without replications.

Checking the obtained estimating method

In this study three experiments of varieties with different features were selected, the method of cropping by estimation was applied and the harvest was

TABLE 2. Combinations of sample sizes starting from the 144 stalks of each lot.

1. No.	Combinations	Abbreviations	Number of stalks per combination
1	12 Vertical x 12 horizontal	12 V x 12 H	144
2	10 vertical x 10 horizontal	10 V x 10 H	100
3	2 vertical	2 V	75
4	2 horizontal	2 H	72
5	3 vertical	3 V	48
6	3 horizontal	3 V	48
7	4 vertical	4 V	36
8	4 horizontal	4 H	36
9	5 vertical	5 V	20
10	5 horizontal	5 H	20
11	6 vertical	6 V	24
12	6 horizontal	6 H	24
13	2 vertical and 3 horizontal	2 V and 3 H	24
14	3 vertical and 2 horizontal	3 V and 2 H	24
15	3 vertical and 4 horizontal	3 V and 4 H	12
16	4 vertical and 3 horizontal	4 V and 3 H	12
17	2 vertical and 6 horizontal	2 V and 6 H	12
18	6 vertical and 2 horizontal	6 V and 2 H	12
19	4 vertical and 6 horizontal	4 V and 6 H	6
20	6 vertical and 4 horizontal	6 V and 4 H	6
21	2 vertical and 12 horizontal	2 V and 12 H	6
22	12 vertical and 2 horizontal	12 V and 2 H	6
23	3 vertical and 12 horizontal	3 V and 12 H	4
24	12 vertical and 3 horizontal	12 V and 3 H	4
25	4 vertical and 12 horizontal	4 V and 12 H	3
26	12 vertical and 4 horizontal	12 V and 4 H	3
27	6 vertical and 12 horizontal	6 V and 12 H	2
28	12 vertical and 6 horizontal	12 v and 6 H	2
29	12 vertical	12 V	12
30	12 horizontal	12 H	12
31	10 vertical	10 V	10
32	10 horizontal	10 H	10

performed by weighing on each experimental plot. The two values (estimated and actual) obtained were evaluated through a variance analysis of twofold classification with replications.

RESULTS

It was found that there were no significant differences among the four shapes of plots under study It proved useful to use the latter as replications in the analyzed areas.

Highly significant differences were evident among the areas under study and when the Duncan test (5%) was applied, it was found out that those areas of 60 m^2, 80 m^2 and 120 m^2 did not integrate homogeneous groups with the area showing the least standard error (100 m^2); the rest of the areas did not show

significant differences with it. Among the areas that provided better results, the ones of 30 m^2, 40 m^2, 50 m^2, 100 m^2 and 150 m^2 were selected to correspondingly study replications.

For the area of 30 m^2: the following results were obtained 13 to 14 replications in cane plant, 19 to 20 in first ratoon; regarding the two stubs together, 21 significantly different replications were obtained. In the case of 40 m^2 for cane plant the number of replications was from 17 to 18, for first ratoon they were 5 to 6, and for plant and first ratoon together the number of replications was 8. For a 50 m^2 area the number of replications in cane plant was 7 to 8, in first ratoon 5 to 6, and in both stubs, 8. For 100 m^2 the number of replications in cane plant was 3 to 4, in first ratoon was 3 to 4, and the two stubs together, 3. In this case significant differences were appreciated among the replications.

For 150 m^2 there were no significant differences in cane plant: in first ratoon the number of replications was 5 to 6 and in the two stubs the number was 4. All these results are shown in Table 3. From the table it can be seen that the better areas were those of 50 and 100 m^2, although the last area showed differences among the replications and comprises a larger area to be analyzed.

As to type of stool, final results showed that there were no significant differences between the two types of stubs analyzed.

Inner border effect varieties

In the corresponding study of cane plant, pertinent differences among the furrows were not obvious; in the case of groups of furrows the same situation prevailed for the five parameters analyzed: Brix in %, % of Pol in juice, cane in stalks/furrow at 12 months and stalks/furrows at 6 months (Fig. 1).

In the comparative analysis with 12 varieties great differences were not evident neither among the furrows nor in the group of furrows for the varieties in the case of cane kg/furrow parameter (Fig.2). For this reason it is concluded that in the studied varieties and under present conditions, the so-called border effect is not present.

Size of the samples for sugar analysis

The variance for each combination of the three analyzed factors (varieties, time of sampling and size of the samples) was determined in terms of % of Brix, % of Pol in juice and weight/stalks in kg. A variance analysis of threefold classification was applied over them and it was found out that significant differences among the three varieties and the times of sampling, in cane plant as well as in first ratoon. This made possible to work with all data together for each combination and with the means of the variances so obtained, a variance rate analysis was applied.

In this way it could be determined that in all the cases two highly-significant decreases in variability were observed due to the size of samples. The first decrease was noticed when the samples had two stalks and the second when the samples ranged between 4 and 6 stalks (Tables 4 and 5; the results of % of Pol

TABLE 3. Behavior of the best areas and number of replications in the cane kg/plots parameter.

Type of area	Number of replications per type of stubs		
	Plant cane	1st ratoon	Plant cane – 1st ratoon
30 m^2 (2 in 2)	13 – 14	19–20*	
30 m^2 (1 in 1)			21*
40 m^2 (2 in 2)	17–18	5–6	
40 m^2 (1 in 1)			8
50 m^2 (2 in 2)	7–8	5–6	
50 m^2 (1 in 1)			8
100 m^2 (2 in 2)	3–4	3–4	
100 m^2 (1 in 1)			3*
150 m^2 (2 in 2)	NS	5–6	
150 m^2 (1 in 1)			4

* **Variants with significant differences among replications*

in juice and weight/stalks in kg are not shown because they are similar to those of the Brix in %, in cane plant as well as in first ratoon). It was concluded that the optimum size of samples is 5 canes since in all the cases this size provided good results.

Variability among and within the stools

While evaluating the final results it was determined that in any case there were pertinent differences among the stalks of the same stool. However, in repeated instances significant differences were found among the stools (Table 6). Consequently, it is concluded that it is necessary to pick stalks from different stools at random to obtain a truly representative sample.

Extent of the obtained method of estimation

While appraising final results it can be verified that there were no great differences between the final estimating method and the harvest by weighing in any

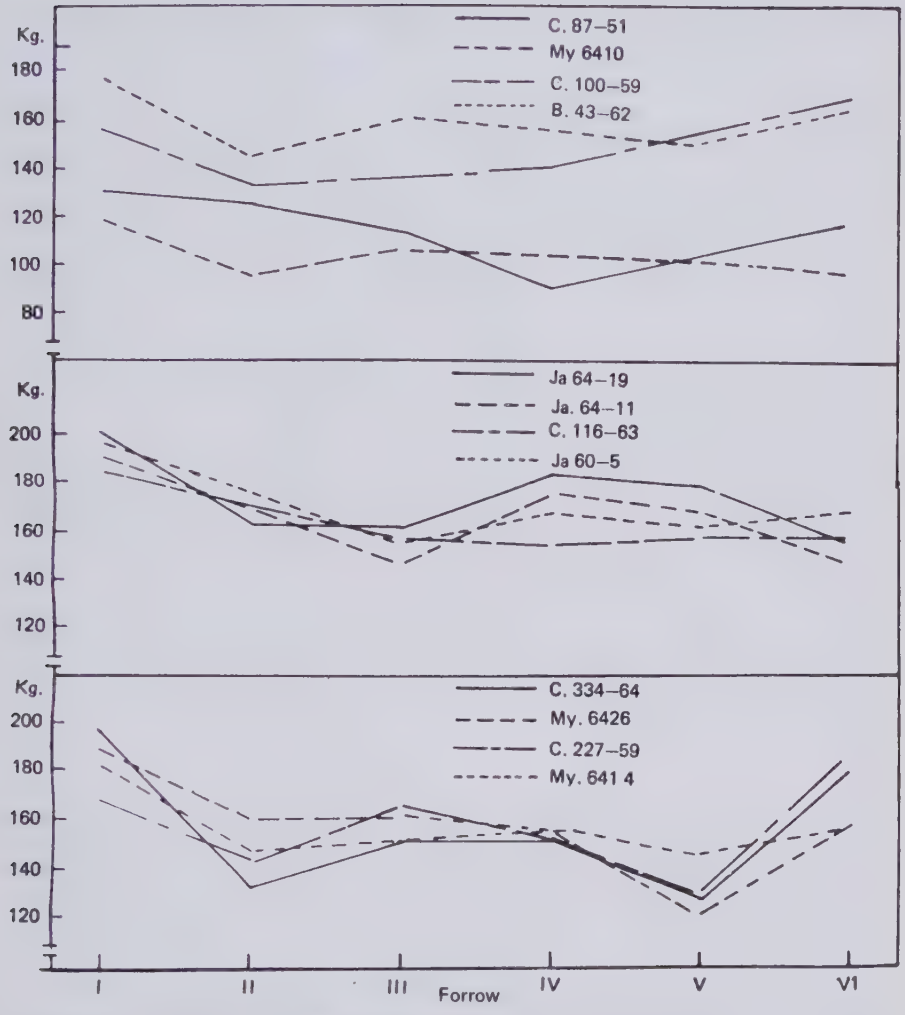

FIGURE 1. Results of inner border effect on experimental plots of six furrows, with three varieties of sugarcane.

of the three selected experiments of varieties. It follows then that the suggested method for computing cane yield in experimental plots can replace the harvest by weighing of the whole plot.

CONCLUSIONS

The type of stub has no effect on the area, shape and number of replicates of the plots. Working with plots having 3 to 6 furrows does not bring about great differences among their shapes. The optimum area of the experimental plots is 50 m^2 with 5 to 8 replications. Varieties involved in these studies showed no effect due to inner borders, both in the plant and in first ratoon. Inner borders of experimental plots can be cut out, in the comparative studies of varieties, when-

TABLE 4. Results of the f test (Rate of variance) applied to the variability of Brix in 1 % of plant cane.

No.	Combinations	$s^2 . 10^2$	n	$s^2 x_1 / s^2 x_2$	Significance
1	10 V x 10 H	107.93	100	1.004	—
2	12 V x 12 H	107,50	144	1.69	N. S.
3	2 H	63.86	72	1.01	**
4	2 V	63.13	72	1.21	N. S.
5	3 V	53.08	48	1.21	N. S.
6	3 H	59.05	48	1.63	N. S.
7	4 V	36.89	36	1.73	N. S.
8	4 H	32.22	36	1.96	**
9	5 H	28.16	20	2.27	*
10	33 V and 2 H	26.91	24	2.38	**
11	2 V and 3 H	26.48	24	1.02	N. S.
12	5 V	25.97	20	1.04	N. S.
13	6 V	24.38	24	1.10	N. S.
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
32	12 V and 6 H	1.45	2	18.56	N. S.

* *Significant differences at 5%*
** *Significant differences at 1%*
N.S. No significant differences

TABLE 5. Results of the F test (Rate of variance) applied to the variability of Brix in % in frist ratoon.

No.	Combinations	$s^2 . x 10^2$	n	$s^2 x_1 / s^2 x_2$	Significance
1.	10 V x 10 H	181.21	100		—
2	12 V x 12 H	171.74	144	1.05	N. S.
3.	2 H	93.13	72	1.9	**
4.	2 V	89.39	72	1.04	N. S.
5.	2 H	66.00	48	1.41	N. S.
6	3 V	65.01	48	1.43	N. S.
7	4 H	53.25	36	1.74	**
8	5 H	44.34	20	2.10	*
9	4 V	43.25	36	2.15	**
10	5 V	40.16	20	1.08	N. S.
11	6 H	39.47	24	1.10	N. S.
12	6 V	34.33	24	1.26	N. S.
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
32	6 V and 12 H	2.13	2	20.32	N. S.

* *Significant differences at 5%*
** *Significant differences at 1%*
N.S. No significant difference

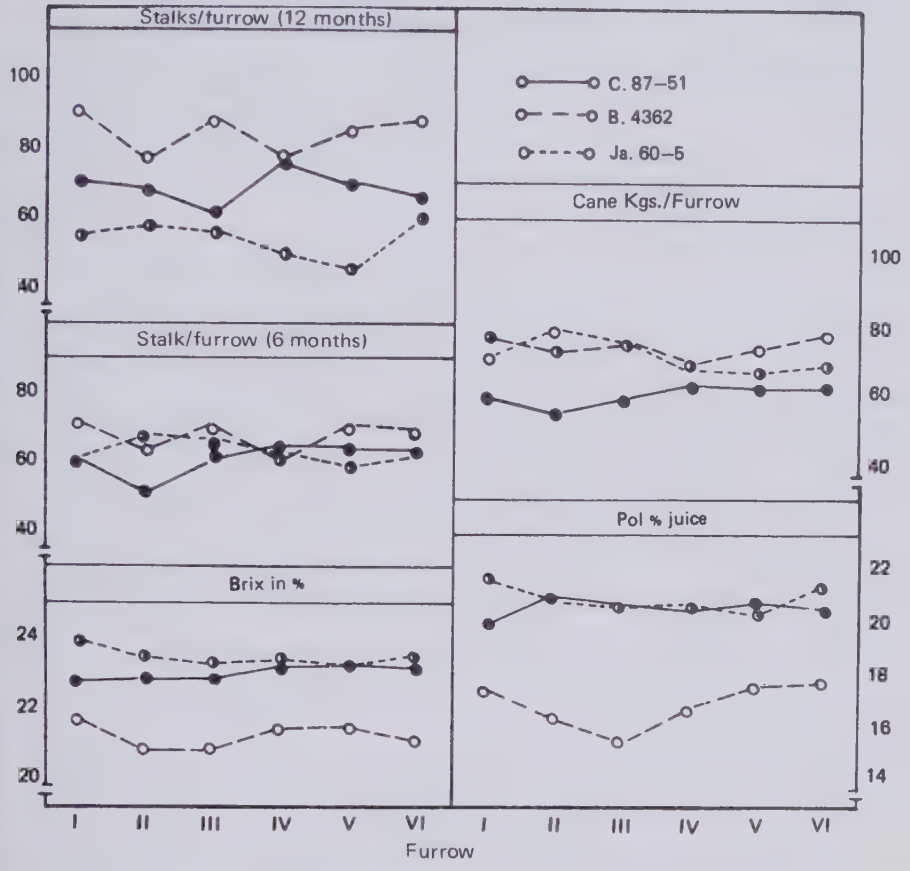

FIGURE 2. Behavior of inner borders of the experimental plots in twelve varieties of sugarcane on 1st ratoon (kg of cane per furrow).

ever the two furrows of outer borders are kept around the experimental block and a no planted furrow is left between adjoining plots.

Random sampling per stalks within the experimental plot provides a more representative variability of the population than doing so in complete stools. The sampling of sugarcane stalks for sugar analysis and computation of weight should be carried out by taking 4 samples of 5 stalks each per experimental plot. To appraise cane yield on plots of 4 furrows able to be cropped it is possible to use the formula

$$P_p = \frac{T_c \times P_m}{K}$$

TABLE 6. Summary of the importance of the variability within and among the stools for the varieties: C 87–51, C 353–69 and C 568–69.

		1st Sampling		2nd Sampling		3rd Sampling	
Types of stubs	Variation	Brix	Pol	Brix	Pol	Brix	Pol
Plant cane	$E._{p.}$					N. S.	N. S.
C87–51	$D._{p.}$					N. S.	N. S.
First ratoon	$E._{p.}$	N. S.	N. S.	N. S	*	N. S.	N. S.
C 87–51	$D._{p.}$	N. S.	N. S.	N. S.	N. S	N. S	N. S.
Plant cane	$E._{p.}$					*	*
C 353–69	$D._{p.}$					N. S.	N. S.
First ratoon	$E._{p.}$	N. S.	N. S.	N. S.	N. S.	N. S.	N. S.
C 353–69	$D._{p.}$	N. S.	N. S.	N. S.	N. S.	N. S.	N. S.
Plant cane	$E._{p.}$					N. S.	N. S.
C 568–69	$D._{p.}$					N.S.	N. S.
First ratoon	$E._{p.}$	N. S.	N. S.	N. S.	N. S.	N. S.	N. S.
C 568–69	$D._{p.}$	N. S.	N. S.	N. S.	N. S.	N. S.	N. S.

* *Significant differences at 5%*

N.S. *No significant difference*

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METODOLOGIA EXPERIMENTAL PARA LA INVESTIGACION DE CAMPO CON VARIEDADES DE CAÑA DE AZUCAR EN CUBA.

N. M. Ramos y J.L. Perez

RESUMEN

Se presentan en este trabajo los resultados de una serie de investigaciones sobre metodologia experimental en caña de azucar. Estas investigaciones fueron conducidas por mas de 8 años en el Instituto de Investigaciones de Caña de Azucar en Cuba.

Se analizaron en estas investigaciones diferentes factores que afectan decididamente la confiabilidad y precision de los experimentos de campo con caña de azucar; tamaño y forma de la parcela experimental, numero optimo de replicaciones, efecto de los bordes internos, tamaño y numero optimo de muestras para analisis de azucar y estimaciones de cosecha en areas experimentales en lugar de peso.

Los resultados obtenidos muestran que: (a) en el caso de parcelas con 3 a 6 surcos, no se observan evidentes diferencias entre formas de las mismas; el area optima es de 50 m^2 con 5 a 8 repeticiones, independientemente de la edad del corte. (b) En las variedades analizadas los efectos de bordes internos no son evidentes, de manera que estos pueden eliminarse manteniendo solo los bordes externos del experimento. (c) A efectos de hacer una muestra aleatoria de tallos, 4 muestras de 5 tallos deberian tomarse en cada parcela. (d) El metodo de estimacion del rendimiento puede reemplazar al de pasaje, dandose un formula para aplicarle en tales circumstancias.

CLONAL SELECTION ACROSS ENVIRONMENTS ON SUGARCANE

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ABSTRACT

This paper reports some of the results obtained from sugarcane clonal selection under 4 environments (localities). Repeatability (R) estimates are compared to actual selection experiments in each locality.

Experiments were based on 80 genotypes (clones) selected at random from 4 progenies. Cane yield and pol % juice were controlled in replicated clonal plots (2 line x 5 m length) at plant and stubble crops. Repeatability was estimated through simple correlations, while phenotypic and genotypic correlations were also estimated from variance and covariance analysis. Selections were evaluated by means of the coincidence index (IC%).

Results indicate that repeatability estimates within localities might be strongly influenced by seasons and residual "C" effects. In the case of cane yields, these effects appear to be equal in magnitude. It seems that certain environments tend to favor the selection of wide adaptable types. This was the case under locality D (Famillа), where it was possible to select for cane and better quality than in other localities investigated. The average performance of the clones was also forecasted well from this locality.

Results also suggest that B x E interactions occur throughout the sugar belt in Tucuman. This justifies the practice clonal selection at several localities simultaneously.

INTRODUCTION

Sugarcane is cultivated in Tucuman (Argentina) in a wide area which is quite variable as far as soil and climate is concerned. Soils vary from medium heavy to light, from well drained to flat easy flooding, from very fertile to quite poor and even saline soils. Rains vary from 1200 mm to the West, down to 800 mm or even less to the East. Temperatures are also variable throughout the area.

Inspite of this, sugarcane breeding and selection up to a few years ago was performed at one locality only. This procedure aimed to select wide adaptable types. As it was thought that such a procedure could attempt against the identification of varieties well adapted to limited areas, clonal selection is now being carried out at several localities from the very early stages (Mariotti[6]).

Falconer[2] studied the effects of the selection environments on the expression of traits by the individuals. He proposed a scheme to represent the kind of genotypic and environmental effects that interact in the resemblance between genotypes growing under different environment. If the genotype x environmental interaction is high and the environmental correlation is low, then the efficiency of the selection between environments will be low too. This problem was also discussed by Skinner[7] in connection with sugarcane. These ideas were further worked out by Jinks and Connolly[4] with proposals on the kind of environments needed for the selection of wide adaptable types.

George[3] reported better efficiency in a selection practiced at 2 localities in Mauritius, which have high quality environments. Daday *et al*[1] reported that it was convenient to select for superior lines of alfalfa under intermediate quality environments.

It has been shown in Mauritius[5] report that there was an effect of the locality of selection on cane efficiency. It was also reported that the genotypes may use different mechanisms to express their characteristics under varying environments.

The aim of this paper is to report some of the results obtained in clonal selection experiments with sugarcane at different localities in the sugar belt of Tucuman (Argentina), comparing estimates of Repeatability to the results of actual selection experiments.

MATERIALS AND METHODS

The research was carried out with a group of 80 genotypes (clones) taken at random from 4 original unselected crosses at the Experimental Station. The clones were planted in 4 sites, to represent typical districts of the sugarcane belt. The most important characteristics of these districts are summarized in Table 1.

The genotypes were planted in each site in a randomized block design with 2 replications. Plot size was 2 rows x 5 m length. Controls were done for several yield and quality trials at the time of plant and first stubble crops. Only cane yield and pol % juice were considered for this report.

Sets of data for each condition were paired in order to estimate repeatabilities under different alternatives of years, crops, and locations. Repeatability (R) was estimated by means of the simple correlation coefficient. Intra-class correlations were also estimated from variable analysis. Some genotypic correlations were also estimated from covariance analysis between sites and between crops within localities. The different estimates were at the time

compared to the results of actual selection experiments. A moderate selection pressure (20%) was chosen with this purpose, selection being bidirectional, towards the positive and negative sides of the distribution.

TABLE 1. Predominant soils and some climatic characteristics in the localities investigated

Locality (1)	Predominant Soil Groups (2)	Year (3)	Rains Jan. (mm)	Rains July (mm)	Temperature Jan. °C	Temperature July °C	No. of Frosts (Av/Yr)	Ep (mm)	Water Balance (mm)
A	Mt/Mf	947	187	10	25.3	11.9	3.0	915	+ 32
B	Mx/Mc	737	146	8	27.0	13.6	4.3	1030	−293
C	Mf	983	209	10	25.6	12.8	3.7	1045	+ 52
D	Md/Mq	995	212	4	25.0	11.8	3.5	894	+119

(1) *Localities:* *A* — *Las Talitas* *B* — *Los Ralos* *C* — *Conception* *D* — *Famailla*

(2) *Predominant Soil Groups: (After Zuccardi and Fadda[9], 1972).*

Mt/Mf — *Typical Arguidol; Hapludol Fluventico, cumulico (well drained).*

Mx/Mc — *Typical Arguido; Haplustol cumulico — fluventico*

Mf — *Hapludol fluventico; Hapludol cumutico; Hapludol entico.*

Md/Mq — *Hapludol fluventico acuico. Hapludol cumulico (not well drained); Haplucuol fluventico — cumulico — entico.*

(3) *Climatic data according to Torres Bruchmann[8] (1976, 1977) and Minetti (unpublished data).*

E_p — *Potential Evapotranspiration.*

The efficiency of the selection between and within environments was measured by means of the coincidence in the selection between 2 sets of data ($A \cap B$) to the number of individuals selected in both A or B ($A \cup B$), being expressed as a percentage. The IC values vary from 0 to 1, and for the purpose of this research, it is considered to be associated to the degree of genetic determination and with the repeatability between paired sets.

RESULTS AND DISCUSSION

The selection within sites

Table 2 shows repeatability estimations for cane yield and pol % juice in each locality taking into account different kinds of relationship among sets of data.

As for R estimates between replicates in the same crop (1) it seems that the expressions of cane yield is more repeatable in locations B and D, while the pol % juice repeats better under locality D.

TABLE 2. Repeatability (R) estimations within localities and phenotypic and genotypic correlations between crops within localities

Locality	Cane Yield (1)	(2)	(3)	(4)	(5)	Pol % Juice (1)	(2)	(3)	(4)	(5)
A	.44**	.46**	.17			.71**	.46**	.52**		
	.41**	.46**	.42**	.45	.99	.71**	.74**	.59**	.58	.64
B	.62**	.64**	.41**			.67**	.69**	.65**		
	.51**	.51**	.30**	.47	1.0	—	—	—	—	—
C	.46**	.61**	.58**			.60**	.59**	.61**		
	.51**	.67**	.49**	.64	.59	.56**	.54**	.51**	.55	.58
D	.58**	.52**	.46**			.75**	.72**	.74**		
	.61**	.59**	.39**	.55	.76	.83**	.83**	.68**	.76	1.0

(1) *R estimates between replications within years. In this case values in the upper line refer to plant cane and those in the lower one to stubble crops.*

(2) *R estimates between plant and stubble crops within replications. The two values refer to different replications in the same locality.*

(3) *R estimates between plant and stubble crops between replications (crossed replications).*

(4) *Phenotypic correlation (Intra-class).*

(5) *Genotypic correlation.*

(**) *Significance at the 1% level.*

Comparing plant and stubble crops within replications (2) results show that locality A is a poor repeatable one as in the previous comparison between sets. Locality D appears again as a favorable one to repeat quality (pol % juice). The estimates of repeatability under this sort of information is supposed to involve a residual clonal ("C" effect). Concerning cane yield it seems that on the average the residual "C" effect is comparable in magnitude to the within years effect, except under locality C, where it appears to be larger than the season effect. This "C" effect does not seem to affect juice quality estimates from one year to the next (from plant to first stubble crop).

When considering values estimated for R between replicates between crops (under (3) in the same Table), the estimates were strongly lowered in the case of cane yields, but not in the quality of canes. Estimates of R in this experiment are slightly under previous estimates in replicated plots (0.69 and 0.67), Mariotti[6].

These estimates are supposed to be nearer the true degree of genetic determination (DGD) in the mixed progeny herein investigated. These are comparable to previous DGD estimates in replicated plots (0.39 and 0.35) for cane yield, but overestimated as for pol % juice is concerned (previous estimation for this trait reached 0.38 and 0.41), Mariotti[6].

Based from the results, it can be concluded that the environmental determination through seasonal or clonal effects (it is considered here that "C" effects are a special kind of environmental effect) promotes a relatively important overestimation of genotypic effects through repeatability. Nevertheless, if localities are compared under (3), it still appears that C and D are superior sites for the purpose of cane yield selection, while D presents also some advantages for the selection of quality.

The intra-class phenotypic correlations (4) follows a similar pattern to the R estimates between crops. On the other hand, genotypic correlations (5) for cane yield shows a strong (genotypic + clonal) resemblance in sites A and B, while environmental interactions appear to occur under sites C and D. In concern to pol % juice the higher R estimates are associated to a high degree of genotypic effect between plant and stubble canes in locality D.

Table 3 shows the results of selection experiments in each site. The IC indexes are low for cane yield and low to moderately low for pol % juice. In general the IC% follows a pattern associated (though not strictly) to R estimated before. It seems that locality D is the best suited (amongst the ones being investigated in this research) for the selection of both cane yields and quality.

TABLE 3. The within localities clonal selection efficiency estimated through the Coincidence Index (IC) in clonal plots (IC%)

Locality	Selection within crops %		Selection between crops within Reps %		Crossed Plots between Crops %	
(a) Cane Yield						
	(+)	(–)	(+)	(–)	(+)	(–)
A	23.2	28.0	25.5	28.2	14.6	22.0
B	28.2	33.1	21.1	30.6	25.9	36.2
C	25.9	28.0	36.7	36.2	33.3	28.2
D	34.2	33.5	33.5	30.6	20.8	23.2
(b) Pol % Juice						
	(+)	(–)	(+)	(–)	(+)	(–)
A	36.2	39.3	28.0	39.1	28.0	33.5
B	33.3	45.4	39.1	45.4	23.1	39.1
C	33.3	28.3	28.1	28.1	33.3	28.0
D	44.2	36.2	42.8	48.9	45.4	42.2

(+) *Positive selection.*
(–) *Negative selection.*

The selection between sites

Results on the association between clonal performance in a particular site against the overall performance across sites are summarized in Table 4.

TABLE 4. Correlation coefficients (r) estimated between the performance of the clones under a particular environment and the average performance across environments.

Locality	Cane Yield		Pol % Juice	
	Plant	Stubble	Plant	Stubble
A	.50**	.64**	.73**	.82**
B	.59**	.60**	.72**	.83**
C	.71**	.72**	.73**	.75**
D	.69**	.70**	.79**	.88**

(**) *Significance at the 1% level*

As a whole, the stubbles tend to represent better average performance across environments than the plant crops. Juice quality can be forecasted far more precisely than cane yields from almost any single site. While most sites seem to be effective in forecasting the pol % juice, sites C and D in both plant and stubble crops appear to be better forecasters for cane yields than sites A or B.

Table 5 shows several associations between localities as well as the results of selection experiments between them. It can be seen that neither R estimates nor the intra-class correlations show clearly any sort of preferential association among sites. The genotypic correlations however, show this sort of preferences, even though, determination are less important than the ones encountered for the witnin sites associations each time that year or clonal effects were involved. In the case of cane yields however, the intersites determinations are quite similar to the ones found within sites when they were free of those environmental effects. This suggest that the kind of variation occurring between seasons within sites is comparable to that occurring between sites. As to pol % juice, the between sites variation leads to a reduced determination in relation to its equivalent within sites.

On the average, it seems that locality D, and to a lesser extent locality B too, would permit a better identification of genotypes with a wide adaptability as far as cane yields is concerned. This seems to be reasonable since locality D is almost in the proper geographic center of the Tucuman sugar belt. The types of lines selected in this kind of environments should lead, according to Jinks and Connoly[4] to a satisfactory average performance over a wide range of environments. This estimated behavior however, was not actually confirmed strictly through the results of selection experiments as can be seen in the same table.

It can also be observed that the negative selections appear to be more effective than the positive ones (about 20% more effective on the average) in the case of cane yields. This fact might be associated to the concept of "conditional" heritability discussed in a previous report, where evidences of

TABLE 5. Repeatability, phenotypic and genotypic correlations estimated between localities (averages of plant and stubble crops). Coincidence indexes (IC%) between localities

Locality		Cane Yield B	Cane Yield C	Cane Yield D	Pol % Juice B	Pol % Juice C	Pol % Juice D
A	(1)	.36**	.38**	.32**	.56**	.56**	.67**
	(2)	.34**	.38**	.27*	.39**	.52**	.64**
	(3)	.97	.34	.66	.68	.90	1.0
	(4)	19.2	19.7	19.3	25.8	31.9	32.7
	(5)	26.7	26.6	22.8	29.4	28.2	38.2
B	(1)		.37**	.42**		.62**	.62**
	(2)		.36**	.35**		.44**	.44**
	(3)		.21	.89		.55	.82
	(4)		19.5	25.1		30.9	35.3
	(5)		25.5	27.4		32.7	33.5
C	(1)			.44**			.58**
	(2)			.41**			.57**
	(3)			.78**			.71**
	(4)			19.7			29.0
	(5)			25.4			39.1

(1) *Repeatability estimates through simple correlations.*
(2) *Intra-class phenotypic correlations.*
(3) *Genotypic correlations.*
(4) *Coincidence indexes (IC%) after a positive selection.*
(5) *The same as (4) after a negative selection.*
(**) *Significance at the 1% level.*
(*) *Significance at the 5% level.*

its occurrence in important yield components as number of stalks, were found (Mariotti[6]).

In view of the relatively low IC% values obtained from selection experiments, it was decided to investigate the efficiency of selection when it is based on more than a single observation per plot (as for example, 2 replicates in a season, or 2 crops in a plot, or both of them). As it was expected to occur, IC values increased with this new approach, from 21 to 39% for cane yields, and from 18 to 35% for pol % juice. From this it can be inferred that part of the differences between sites can be explained through the kind of differences occurring within a site. In other words, the selection between sites could be more effective if it were based on more than a single observation per site.

CONCLUSIONS

From the results discussed in the previous section, the following conclusions can be drawn.

(1) Cane yield repeatabilities within sites are strongly affected by seasonal and residual clonal effects. These effects do not seem to affect R estimates for pol % juice. When seasonal or residual effects are at work as seem to be the case with cane yield (and for sure some of its components too), R estimates not considering those effects are of limited value as a guidance for selection.

(2) Apparent selection efficiency within certain sites might be influenced by environmental components of the sites. Once these effects are controlled however, it is still possible to detect efficient selection sites. Under this experiment site D showed advantages for the selection of both cane and yield quality.

(3) The overall performance of a clone in different environments is forecasted better for pol % juice than for cane yields, which is consistent with DGD estimated for those traits. The overall performance in respect to cane yields can be forecasted better from stubbles than from plant canes, which agree with previous findings in Tucuman. In these experiments, site D forecasted well the average performance of the clones in a wide range of environments.

(4) Genotypic correlations estimated between sites, permitted to detect preferential resemblance between sites. This would help in cases of crossed selection experiments approaches.

(5) Results of crossed selection experiments between sites revealed a rather poor matching between localities. It is important on the one hand, to consider the feasibility of indirect selection approaches through yield components on the other hand, this also suggests the occurrence of a high degree interaction between genotypes and environments in Tucuman, which justifies the increase of selection sites in looking for both wide and narrow adaptability. This procedure is expected to increase the frequency of selections, and the change for further advances in this field.

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SELECCION CLONAL A TRAVES DE AMBIENTES EN CAÑA DE AZUCAR.

Jorge A. Mariotti

RESUMEN

Se informan los resultados obtenidos de experimentos de seleccion de caña de azucar en 4 ambientes (localidades). Se comparan estimaciones de Repetibilidad (R) con resultados de selecciones practicadas en cada localidad.

Las estimaciones y los experimentos de seleccion, se realizaron con 80 genotipos (clones) de 4 progenies sin seleccion previa. En parcelas clonales replicadas (2 lineas x 5 m) se efectuaron controles de rendimiento de caña y pol % jugo, en caña planta y en primera soca. Las repetibilidades se estimaron por medio de coeficientes de correlacion simple. Tambien se estimaron correlaciones intraclase y correlaciones genotipicas a partir de analisis de varianzas y convarianzas. Las selecciones practicadas se evaluaron por medio de los indices de coincidencia (IC%).

Los resultados indican que las estimaciones de repetibilidad dentro de localidades pueden ser muy afectadas por efectos esta-

cionales o por efectos residuales ("C"). Ambos tipos de efectos parecen ser equivalentes en magnitud en el caso de rendimiento de caña. Al parecer la seleccion puede realizarse mas eficientemente desde ciertos ambientes si el objetivo es el de seleccionar variedades de amplia adaptabilidad. Este fue el caso de la localidad D (Famailla) en estos experimentos, la que resulto de entre las investigadas, la mas eficiente para seleccionar por rendimiento y calidad, pronosticando ademas satisfactoriamente el comportamiento general de los clones a traves de las otras areas.

Los resultados sugieren la ocurrencia de interacciones G x A en el area cañera de Tucuman, lo que justifica que la seleccion clonal se ejecute en varias localidades simultaneamente.

THE GENOTYPE-ENVIRONMENT INTERACTION IN EXPERIMENTS OF SUGARCANE VARIETY TRIALS *(SACCHARUM SPP.)*: COMPARISON OF THREE STABILITY METHODS

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ABSTRACT

The genotype-environment interaction (g x e) for yield and brix in eight sugarcane variety trials with 20 genotypes was calculated. The trials were carried out in two localities during three harvest periods.

A statistically significant interaction, g x e, in both localities was observed. All data were analyzed by 3 methods in order to determine stability and/or adaptability. The methods were: linear regression, ecovalence method and the coefficient of determination. All the methods used verified in its own way the discrimination of genotypes by their stability in relation to environment changes.

Finally, the Kendall Coefficient of Correlation was calculated among all stability parameters used verifying positive and significant correlation between ecovalence and linearity deviation, ecovalence and coefficient of determination, and coefficient of determination and linearity deviation.

INTRODUCTION

The genotype-environment interaction in plant breeding had constituted an important problem with regards to the classification of the genotypes when tested in different environments (Comstock and Moll[5]). The first attempts of breeders were directed to reduce or minimize this interaction (Sprague[13]). However, those approaches had as a general characteristic, the statistical use of the g x e interaction, such as the efforts made by Horner and Frey.[8] The first attempt to use the g x e interaction in a dynamic way, was made by Finlay and Wilkinson.[6] It was extended by Eberhart and Russell[3] in the so-called linear regression approach, which finds its origin in Yates and Cochran.[15] There were other approaches, such as Wricke's,[14] Bucio Alanis,[2] and recently, Bilbro and Ray's,[1] which used other parameters to measure stability.

Hill[7] had carried on an interesting discussion of the methods to estimate some parameters of environmental stability. Skinner[10] had already pointed out the necessity of testing the linear regression technique in sugarcane. In this work we want to compare three of these methods, using sugarcane as experimental material.

MATERIALS AND METHODS

Experimental procedure

Eight experiments grouped into two (four experiments each) with twenty genotypes planted in two zones in the western part of Cuba (Table 1) were used. The original experiments had a randomized complete block design with four replications, having 1.6 m. between rows, each row being 15 meters long, and five rows making a plot. Both localities have red ferralitic soils with different fertility levels.

TABLE 1. Genotypes used in each experiment. Arrangement of the experiment in groups:

Groups	1	2
Localities	Bauta and Bainoa	Bauta
Experiments	1, 2, 3 and 4	5, 6, 7 and 8
Genotypes	1. My 5354 2. My 5514 3. Ja 60-5 4. C 87-51 5. B 4362 6. CP 5243	1. My 54121 2. My 5596 3. My 5346 4. My 5353 5. C. 89-51 6. C. 87-51 (T) 7. Pomex 72 8. My 5723 9. My 5364 10. My 54142 11. My 53172 12. B 41227 13. My 54130 14. My 56166

The experiments were analyzed from data taken from three harvests, so that 12 environments could be made in each group (four experiments and three harvests).

Statistical procedure

After making a factorial analysis in both groups in order to prove the effect of the g x e interaction, three different stability models were applied to the yield and brix.

Model I

The so-called environmental index (Ij) was estimated in both groups of experiments, according to Eberhart and Russel [3].

$$Yij = \mu + \beta ilj + \pounds ij$$

Therefore, the two stability parameters, *bi* and *£ ij,* were estimated and tested for each genotype in the appropriate way.

Model II

Wrick's model[14] for estimating the value of each genotype in its participation in the g x e interaction *(Wi),* was used testing it in the way proposed by him. Varieties having a high value are considered unstable, while those having less *Wi* value are considered more stable.

Model III

The *bi* value was estimated in this model in a way similar to that found in Model I. As a second stability parameter, the value of the coefficient of determination (r^2) between the environmental index *(Ij).* and the value of each genotype in that environment was estimated. A variety was considered more stable in the same measure as its r^2 value was statistically higher than the tester. The r^2 value was tested according to Sokal and Rohlf [11].

Finally, the three models were compared using Kendall's coefficient of rank correlation (τ) to all the stability parameters used to estimate the relative stability of the varieties.

RESULTS AND DISCUSSION

Fig. 1 shows the results of the applications of Model I for yield. In this group, varieties 3, 4 5 and 6 may be considered as stable varieties. Variety 2 proved to be unstable (bi = 1.39). Our results agreed with those of Campo, Morales and Perez[4] in relation to the fact that this variety deteriorated under conditions where there were no irrigation.

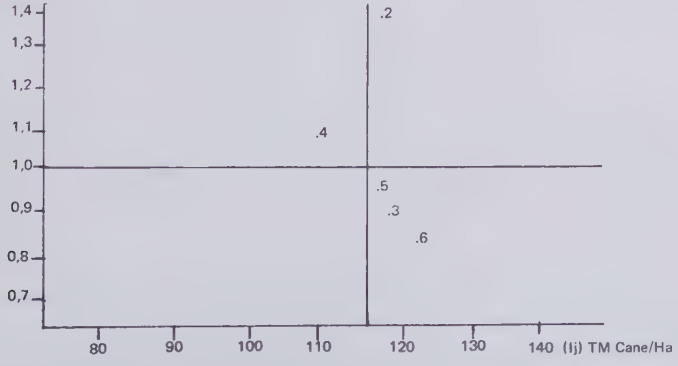

FIGURE 1. Relationship between Yield and Stability. Group 1.

In Table 2 we may observe the result of the variance analysis of yield for both groups. In both cases there were significant differences for the genotypes as well as for the g x e interaction, which fulfills the necessary premise for the application of the stability models. Similar results were obtained for brix (Table 3).

TABLE 2. Variance analysis for t of cane/ha

	Group 1			Group 2	
Source of Var.	D. F.	M. S.	Source of Var.	D. F.	M. S.
Genotypes (A)	5	813.74**	Genotypes (A)	13	5107,10**
Environments (B)	11	28945.86**	Environments (B)	11	84228.72**
A x B	55	778.24**	A x B	143	12132.53**
Error	216	187.40	Error	501	749.27

** $P < 0.01$

TABLE 3. Variance analysis for total solids (Brix)

	Group 1			Group 2	
Source of Var.	D. F.	M. S.	Source of Var.	D. F.	M. S.
Genotypes (A)	5	6.05**	Genotypes (A)	13	51.51**
Environments (B)	11	53.26**	Environments (B)	11	316.10**
A x B	55	3.33**	A x B	143	37.78**
Error	216	1.25	Error	501	1.44

**$P < 0.01$

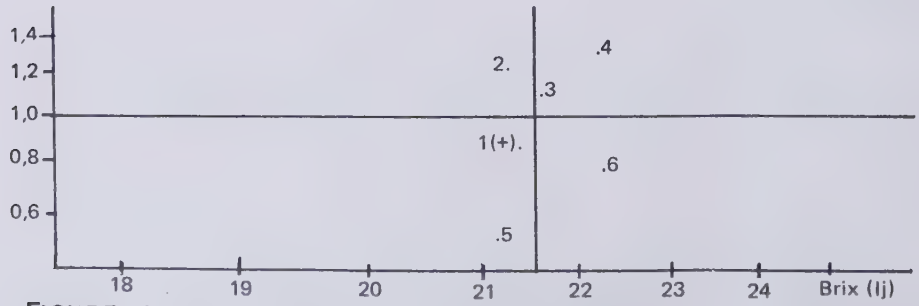

FIGURE 2. Relationship between Brix and Stability in Group 1. Varieties with (+) mean significant with $p < 0.05$.

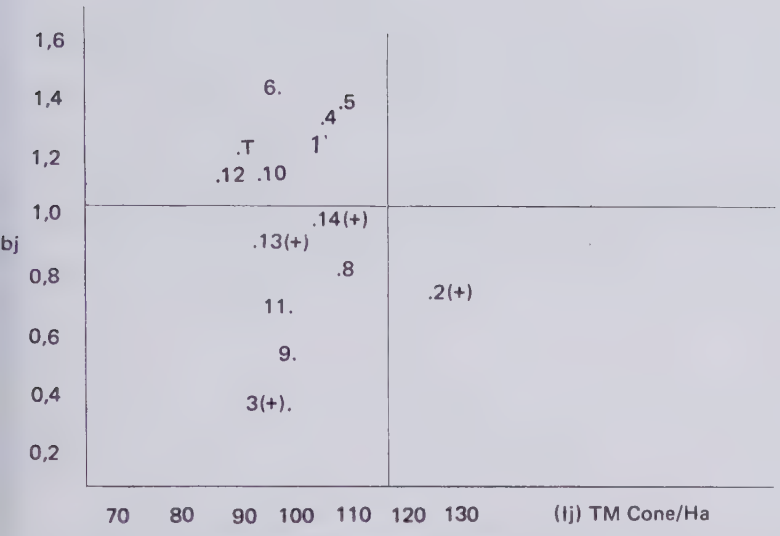

FIGURE 3: Relationship between Yield and Stability in Group 2. Varieties with (+) mean significant with $p < 0.05$.

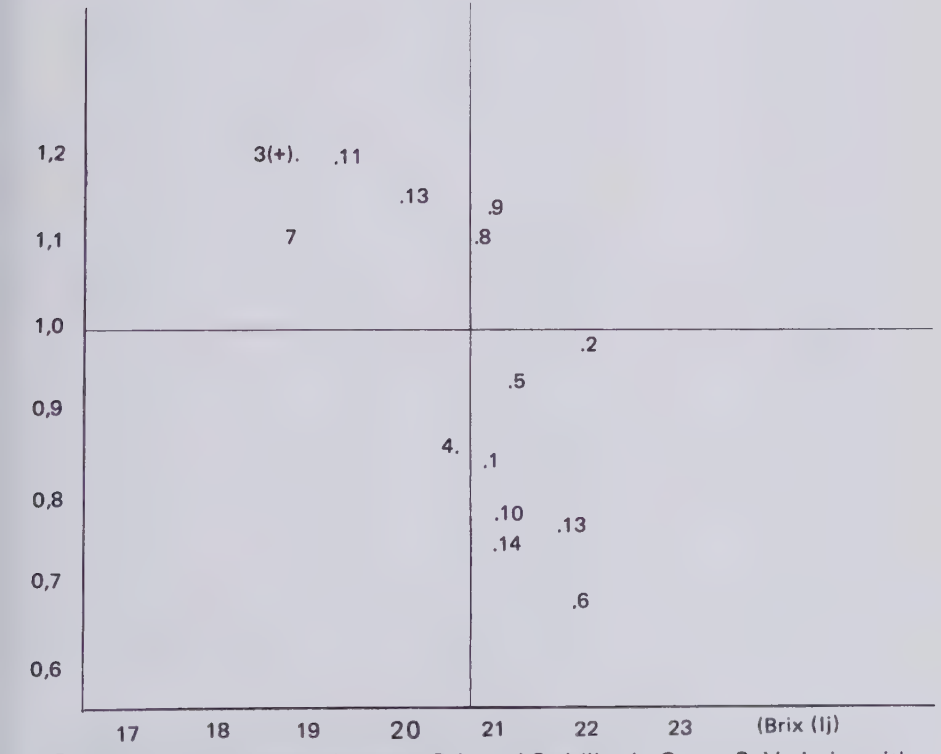

FIGURE 4: Relationship between Brix and Stability in Group 2. Varieties with (+) mean significant with $p<0.05$.

Fig. 2 shows that only the Ja-60-5 (3) variety fulfilled the two stability parameters and had a mean slightly higher than the general mean for brix. Fig. 3 shows that six outstanding varieties met the two stability parameters although only two of them (1 and 8) were higher than the general mean.

Fig. 4 shows that there were nine varieties which fulfilled the two stability parameters, while only four were higher than the general mean (1, 2, 5 and 8). In Fig. 4, we may observe the stability analysis for the characters which were analyzed by the ecovalence method. It should be pointed out that the method discriminated the performance of the varieties in different environments for both characters in both groups of experiments.

TABLE 4. Ecovalence values (Wi)

		Group 1	
		t cane/ha	
1.	My 5354	1570.85 ab (*)	14.95 a
2.	My 5514	2441.35 a	3.68 bc
3.	Ja 60-5	867.73 abc	6.78 abc
4.	C 87-51	763.81 bc	4.92 bc
5.	B 4362	1003.74 abc	8.51 ab
6.	CP 5243	536.08 abc	2.83 c
		Group 2	
1.	My 54121		
1.	My 54121	3276.93 abcd	7.30 ab
2.	My 5596	6108.78 ab	3.20 bcd
3.	My 5346	9146.85 a	11.20 a
4.	My 5353	4468.41 abc	3.98 abcd
5.	C 89-51	3843.11 abc	1.76 cd
6.	C 87-51	2826.84 bcd	8.58 cd
7.	Pomex 72	6557.39 abc	7.25 ab
8.	My 5723	4709.95 abc	8.10 ab
9.	My 5364	4225.29 abc	1.55 a
10.	My 54142	1288.51 d	5.21 abc
11.	My 53172	3233.51 abcd	5.42 ab
12.	B 41227	1839.25 cd	5.25 ab
13.	My 54130	4108.12 abc	5.34 ab
14.	My 56166	3665.30 abcd	4.52 abcd

Data with different letters differ statistically ($P < 0.05$).

Table No. 5 shows the results of the stability analysis using the r^2 value. We may also observe that it was possible to discriminate the varieties in different stability groups with this method.

TABLE 5. Values of "Coefficient of Determination" (r^2)

	Variety	t cane/ha	Brix
		Group 1	
1.	My 5354	0.7839†	0.6430†
2.	My 5514	0.9198	0.9771
3.	Ja 60-5	0.8585	0.8697
4.	C 87-51	0.9227	0.8826
5.	B 4362	0.8684	0.7494
6.	CP 5243 (T)	0.9607	0.9166
		Group 2	
1.	My 54121	0.0009	0.8390
2.	My 5596	0.5789†	0.9306††
3.	My 5346	0.3043†	0.8727
4.	My 5353	0.9017	0.9135††
5.	My 89-51	0.9325	0.9617††
6.	My 87-51 (T)	0.9846	0.5033
7.	Pomex 72	0.9484	0.8945††
8.	My 5723	0.8452†	0.8695
9.	My 5364	0.6874†	0.9886††
10.	My 54142	0.9318	0.8894
11.	My 53172	0.7588†	0.9422††
12	B 41227	0.9112	0.9471††
13.	My 54130	0.7229†	0.9138††
14.	My 56166	0.7696†	0.9459††

† r^2 differs from standard ($P < 0.05$)
†† r^2 differs from standard being higher in stability ($P < 0,05$)

TABLE 6. Matrix of "Kendall Range Correlation" (τ)

	t cane/ha				Brix			
	Wi	bi	£ij	σ^2	Wi	bi	£ij	σ^2
	1	0.50	0.86	-.60	1	0.20	0.86*	0.86*
Wi	1	0.30	0.38	0.26	1	0.12	0.74*	0.69*
	–	1	0.33	0.10	–	1	–0.20	–0.20
bi	–	1	0.321	–0.74*	–	1	0.15	0.07
£ij	–	–	1	0.20	–	–	1	0.86*
			1	0.60	–	–	1	0.85*
σ^2	–	–	–	1	–	–	–	1
				1				1

Upper values belong to Group 1 of experiments and the lower ones belong to Group 2 of experiments

** Significant differences ($P < 0.05$)*

The results of the estimates of the rank correlation (τ) appear in Table 6. A high positive correlation betwen the parameters ecovalence (*Wi*) and linear deviation ($\pounds$ ij) were shown. However, analyzing the other stability parameter in Model I (*bi*) in relation to Wi, we see that there was a tendency to a positive but insignificant correlation in both groups. Similar results were obtained by Luthra and Singh[9] with eight wheat varieties in 24 environments.

A high positive correlation was also found between the Wi and r^2 ranks for brix in both experiment groups.

According to our results, it would be possible to establish the variety rank for yield using Wi and $\pounds$ij indistinctly. The parameters Wi and $\pounds$ij may be used for brix.

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LA INTERACCION GENOTIPO-AMBIENTE EN EXPERIMENTOS CON VARIEDADES DE CAÑA DE AZUCAR *(SACCHARUM SPP.)*: COMPARACION DE TRES METODOS PARA INVESTIGAR ESTABILIDAD.

G. Galvez

RESUMEN

Se calculo la interaccion genotipo-ambiente para rendimiento de caña y brix en 8 experimentos con variedades que incluian 20 genotipos. Los experimentos fueron conducidos en dos localidades durante 3 estaciones de cosecha.

Se observo una interaccion genotipo y ambiente significativa en ambas localidades. Los datos fueron analizados por 3 metodos diferentes para determinar estabilidad y/o adaptabilidad. Los metodos fueron: "Regression Lineal", "Metodo de las ecovalencias" el "Coeficiente de determinacion" (Bilbro y Ray, 1976).

Todos los metodos investigados verifican de una manera particular la discriminacion de genotipos por estabilidad en relacion con los cambios ambientales.

El coeficiente de correlacion de Kendall se calculo para asociar todos los parametros de estabilidad, observandose correlaciones positivas y significativas entre "Ecovalencia" y "Desviaciones de la linearidad", "Ecovalencia" y "Coeficientes de determinacion" y "Coeficientes de determinacion" y "desviacion de la linearidad".

STUDY OF GENOTYPE – ENVIRONMENT INTERACTION IN SUGARCANE. I. THE INTERACTION OF THE GENOTYPES WITH PLANTING DATES AND HARVEST CYCLES

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ABSTRACT

Fourteen cultivars of sugarcane in two harvest cycles (12-14 months and 16-17 months) and two planting dates (October and June) were studied using four experiments with plant cane and two ratoons.

The factorial analysis of the results showed statistically significant differences among genotypes for cane t/ha, Pol % in cane, Pol t/ha and the ratio cane t/ Pol t. From the interactions analyzed, those of planting date x harvest cycles proved to be highly significant for all the characters analyzed with value of 61, 89, 26 and 85% of the total phenotypical variation and being 80, 13, 9 and 10 times greater than the variance obtained, due to the effect of the genotypes for the tons of cane/ha, Pol % in cane, the tons of Pol/ha and the ratio cane t/ Pol t, respectively. The most effective combination proved to be that of planting in October with a 16-17 harvest cycle.

The degree of genetic determination (g^2) for the four characters showed that the juice quality and the ratio cane t/ Pol t were the characters with the highest (g^2).

INTRODUCTION

Selection in sugarcane includes two fundamental phases: studies of seedlings and first clonal stages; and selection in the last clonal stages, which may be classified as that of zonal or location trial studies (Skinner[17], Galvez[8]).

During the last location trial stages it is important to find the value of the effect exerted by the so-called environment, and the interrelation between the genotypes and the environmental factors which is sometimes an essential component of the phenotypical variation in genetic experiments. (Comstock and Moll[5]).

In sugarcane, it is important to carry out varietal experiments to find out the value of the g x e interaction in the last stages of selection. In Cuban conditions, values of more than 60% of the phenotype variation have been

reported for yield and some of the components (Galvez, Espinosa and Ortiz[9]), while Mariotti[14] in Argentine, George[10] in Mauritious and Skinner[17] in Australia, have also reported very high values for the g x e interaction. The planting dates and the harvest cycles constitute fundamental factors of the cane environments, as well as localities and years.

This study is part of a group of experiments which have been conducted in the western zone of Cuba with the principal aim of estimating the effects of planting date and harvest cycles on the yield and Pol %, to determine the most efficient combination of planting dates and harvest cycles, regarding the genotypes studied, and also to find out the effect of these factors on the general variability.

MATERIALS AND METHODS

Four experiments with 14 cultivars were planted during two planting seasons and two harvest cycles. The cultivars used were as follows:

My 54121	Pomex 72
My 5596	My 5723
My 5346	My 54142
My 5353	My 53172
C 89-51	B 41227
C 87-51 (S)	My 54130
My 5364	My 56166

Two of the experiments were planted in October, 1972, while the other two in July, 1973, so as to make 4 combinations with the short and medium harvest cycles (13-14 months and 16-17 months).

The experiments were planted in the Sugarcane Station in Bauta, Province of Havana, in deep red ferralitic plastic cells. Each experiment was conducted with the usual agricultural treatment in variety trials (Espinosa[6]) and had no supplementary irrigation.

In each experiment, the cultivars were planted in blocks having 6 rows 15 m long at a distance of 1.6 m between rows, with a randomized block design and 4 replications (Cochran and Cox[4]). The 4 central rows (96 m^2) were used as sampling area. At harvesting time, yield was estimated according to Hogarth and Skinner[11]. The Pol % in sugarcane was estimated before harvesting a sample of 5 random stalks in each block. The other two characters, Pol t/ha and the ratio sugarcane t/ Pol t, were estimated from the above data.

After making three harvests in each experiment, a 14 x 2 x 2 factorial analysis was done and the variance components of each factorial ANOVA were estimated according to the Aleatory Model or Modell II. (Searle[16]).

Starting from the components of variance, the degrees of genetic determination (g^2) defined by Falconer[7] and estimated according to Skinner[17] were estimated from each character analyzed.

RESULTS AND DISCUSSION

Cane t/ha

Table 1 shows the results of the combined variance analysis. With respect to cane t/ha, statistically significant differences (P 0.05) were observed among the genotypes studied, similar to Espinosa's[6] findings.

The planting dates (Winter and Spring) as well as the cycles affected the yield in these experiments, thus showing a distinct advantage in the means of the medium cycles over the short ones, as has been stated by Acosta[1].

The interaction of the genotypes with the harvest cycles and the planting dates was not significant. From the theoretical point of view, it is difficult to explain this result, since the varieties studied were originally selected for short harvest cycles. However, the number of genotypes studied was not wide enough to represent a sample of the original seedling populations from which they were derived.

The interaction between planting date x harvest cycle was highly significant (P 0.01), which shows the marked effect of these factors on yield, which is not a highly heritable character (Hogarth[13], Galvez, Espinosa and Ortiz[9]).

TABLE 1. The 14 x 2 x 2 factorial analysis

Source of variation	Mean squares of the characters analyzed			
	Cane t/ha	Cane Pol %	Pol t/ha	Cane t/Pol t
Genotype (A)	11508.86**	14.07**	419.32**	5.94**
Planting date (B)	424662.16**	3.19	7630.78**	1.29
Harvesting cycle (C)	141283.25**	1.93	3074.85	0.18
A X B	2000.04	1.05	118.72**	1.07
A X C	3087.64	1.06	90.56*	0.87
B X C	923166.46**	187.30**	3973.01**	62.08**
A X B X C	5322.64	1.03	105.15**	1.08
Error	2427.37	1.00	43.76	0.78
C.V. (%)	17.49	7.48	18.72	11.54

*P 0.05
**P 0.01

Pol % in cane

As shown in Table 1, there were highly significant differences among genotypes, which indicate that the juice quality is a characteristic having a high variation and selection possibilities (Brown, Daniels and Latter[2], Hogarth[12]). On the other hand, the fact that there was no difference between the planting dates and the harvest cycles for this character is also understandable, since

it is not highly influenced by environmental conditions, as in the case of Brix, has a high genotypical and phenotypical correlation (Hogarth[13]).

However, the fact that the planting date x harvest cycle interaction is statistically significant should not be surprising, since a particular combination of these factors may change the physiological response of the plant with respect to its sucrose accumulation.

Pol t/ha

This character is of great importance in variety trials; however, its analysis is complex, since it is the product of the characters, each having various components.

The results shown in Table 1 indicate that all the sources of variation were highly significant (P 0.01) and the genotype x harvest cycle interaction only proved to be significant with P 0.05. This also shows that all the factors had marked influence on Pol t/ha under these experimental conditions. However, with such a complex character, it is better to make the genetic selection in these stages through its components.

Relation between cane t/Pol t

Table 1 shows statistically significant differences between genotypes for cane and Pol t, which is a way of expressing the sucrose-converting potentiality of each genotype. Being a characteristic of high heritability in the broad sense (Table 3), it is possible to explain that the planting dates and the harvest cycles are not significantly different. However, the planting date x harvest cycle interaction does present highly significant differences. This result is of great practical and theoretical value, since it revealed that under experimental conditions, at least, one of the ways in which the genotype may express their genetic potentiality is to find its adequate combination, and that the elongation of the cutting cycles improved the results in these experiments.

Degree of genetic determination (g^2) analysis

Table 2 shows the degree of genetic determination (g^2) of the characters analyzed based on replicated blocks. The highest (g^2) values were those of Pol % and that of the relation cane t/ Pol t, which agree with the results obtained

TABLE 2. Degree of genetic determination (g^2)

Character	(g^2) on a replicated plot basis	% σ^2 BC/ σ^2 Phenotypic
Cane t/ha	0.35 ± 0.13	60%
Pol %	0.73 ± 0.12	88%
Pol t/ha	0.67 ± 0.14	25%
Cane t/Pol t	0.75 ± 0.12	84%

by other researchers (Brown, Daniels and Latter[2]; Ladd, Heinz, Meyer and Nishimoto[15]; Galvez, Espinosa and Ortiz[9]).

The (g^2) values for yield was not high, a fact which also agrees with the results found in Literature. In spite of its importance, it did not respond so strongly to selection which may be attributed to the number of stalks which has a low (g^2) value as stated in most reports.

Analysis of the relation among variances

An analysis of the relation among variances was carried out to find out the relative value which the environment, the planting date x harvest cycles interaction and the genotypes had on the environmental variance (Table 3). The results show that the environmental factor (planting dates, harvest cycles and their interaction) proved to be a very high component of the phenotypical variation, reaching values from 87% to 98% in the four characters analyzed.

TABLE 3. Relation between variances

Character	% σ^2 E / σ^2 P	% σ^2 BE / σ^2 P	% σ^2 A / σ^2 P	Relation % σ^2 BC / σ^2 A
Cane t/ha	98%	61%	1%	80.21%
Pol % cane	91%	89%	7%	13.31%
Pol t/ha	95%	26%	3%	9.47%
Cane t/ Pol t	87%	85%	8%	10.45%

CONCLUSIONS

The effects of planting dates and harvest cycles, separately as well as its interaction, proved to have a greater influence than the isolated varietal effect on the variability of the characters analyzed.

It is of great importance to find adequate combination for planting dates and harvest cycles. Under these experimental conditions, the elongation of the cycle with the October planting date proved to be the best combination.

Results show that the Pol % in cane and the ratio cane T/Pol t were the most heritable characteristics which is similar to those reported in international literature. Besides these could serve as basis for future studies in Quantitative Genetics on selection in the final stages of sugarcane under our conditions.

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ESTUDIO DE LA INTERACCION GENOTIPO-AMBIENTE EN CAÑA DE AZUCAR. I. LA INTERACCION DE GENOTIPOS CON FECHAS DE PLANTACION Y CICLOS DE COSECHA.

R. Espinosa y G. Galvez

RESUMEN

Se estudiaron 14 cultivares de caña de azucar en dos ciclos de cosecha (12-14 meses y 16-17 meses) y dos fechas de plantacion (Octubre y Junio), en base a los resultados de caña planta y dos socas.

El analisis factorial de los resultados mostro diferencias estadisticamente significativas entre genotipos para rendimiento de caña, Pol % en caña, toneladas de Pol/ha y la relacion rendimiento de caña/tn Pol. De las interacciones investigadas, las correspondientes a fecha de plantacion y ciclos de cosecha resultaron altamente significativas para todos los caracteres investigados, representando respectivamente el 61, 89, 26 y 85% de las variaciones genotipicas totales, resultando ademas 80, 13, 9 y 10 veces mas grande que las varianzas atribuibles a genotipos. La combinacion mas efectiva resultó la de plantar en Octubre con un ciclo de 16-17 meses hasta cosecha.

Finalmente se analizo el grado de determinacion genetica (g^2) para los cuatro caracteres, observandose que los valores mas elevados corresponden a la calidad del jugo y a la relacion rendimiento de caña/tn de Pol.

SUITABILITY OF AUGMENTED DESIGN IN SUGARCANE BREEDING TRIALS

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ABSTRACT

The comparative efficiency of the augmented R.B.D. and lattice designs for identifying the superior clones of sugarcane breeding experiments was studied in two different sets of genotypes each in two locations over two years at the Sugarcane Breeding Institute, Coimbatore. It was noticed that the top 10% elite genotypes selected on the basis of statistical analysis are almost the same in both the designs. The augmented design, though not equal to lattice design, tries to achieve as much statistical efficiency as possible in its layout and appears to be a handy statistical design for sugarcane breeders who have to evaluate a large number of genotypes as precisely as possible inspite of certain limitations especially in planting material. From the point of view of economy, it can also be considered to be a very useful technique.

INTRODUCTION

In any crop improvement program, efficient breeding methods and screening procedures are necessary and above all, appropriate experimental designs are to be employed to minimize the error component in any field experimentation. Generally, lattice designs enable substantial increases in efficiency in plant breeding trials where the number of varietal entries is large even leading to a gain of 100 per cent on highly heterogeneous soils.

The problem in the case of sugarcane breeding program is that for each seedling being a distinct genotype, often sufficient planting material is not available in initial clonal stages to lay out the evaluation in replicated trials and in the most efficient experimental design. Since each genotypes has material normally to plant in one six-meter row only with about 20 three-budded setts, there is little scope for replication of genotypes. Further the design chosen should allow comparison of a very large number of clones without sacrificing efficiency. Until recently, the Check Plot design with checks at intervals was adopted for clonal evaluation trials in sugarcane.

The augmented design was first suggested by Federer[2] to fulfill the need

for screening new strains of sugarcane and soil fumigants used in growing pineapples. An augmented design is any standard experimental design augmented with additional treatments in the complete blocks, the incomplete blocks, the rows, the columns, etc. (Federer[3]). The idea of utilizing the augmented design started at the Hawaiian Sugar Planters' Association Experiment Station wherein the check plot design was found less unsatisfactory. This design is now being adopted in the sixth stage of screening of seedlings in Hawaii.

This study was undertaken at Sugarcane Breeding Institute, Coimbatore to assess the comparative utility and efficiency of augmented randomized block design with incomplete block designs, namely the rectangular lattice and cubic lattice, during 1977 and 1978 trials respectively, for clonal evaluation trials in sugarcane.

MATERIALS AND METHODS

Two separate locations representing normal sugarcane growing conditions at the Sugarcane Breeding Institute, Coimbatore were selected for this study. During 1977 in one location, 83 clones selected from pre-final clonal trials were planted along with 3 checks and 4 local commercial varieties. In the second location, 86 new clones selected from a different set of pre-final clonal trial were planted in comparison with 4 checks. In both the locations, the augmented randomized block design with 14 blocks as well as the rectangular lattice design of the order of 9 x 10 with two replications were adopted keeping a similar plot size of 2 rows of 6 meters each for every variety (based on the results of UNIFORMITY TRIALS conducted at this Institute). All the genotypes were multiplied earlier for meeting the requirements of planting material for their comparison in the two designs. In the following year, about 210 different sets of clones selected from Second Ground Nursery were planted along with 6 checks in both the locations in Augmented R.B.D. and cubic lattice designs by maintaining a similar plot size of 3 m of single row in both the designs. The rectangular lattice design could be accommodated in each location in an area of about 0.24 ha. and cubic lattice in 0.194 ha. The area required for the corresponding augmented randomized block design was 0.168 ha. and 0.072 ha. in the second location and 0.210 ha. and 0.074 ha. in the first location. Normal and similar cultivation practices were adopted throughout the crop cycle in both locations and lay-outs during both seasons.

Data on germination at 30th day after planting, number of millable canes (NMC) and cane yield in kilograms at harvest were gathered on plot basis. During 1978, data on number of tillers was included in place of cane yield as cane yield for few genotypes could not be obtained due to disease attack. Analysis was carried out as per the methods of Federer[2] and Cochran and Cox[1] for the augmented and lattice designs respectively.

RESULTS

Analysis of variance for the three characters studied in the two different designs in both the locations are presented along with coefficients of variation in Tables 1, 2, 3 and 4. In the augmented design, the error variances and co-

TABLE 1. Analysis of variance for Augmented R.B.D. and Rectangular Lattice Designs for germination, NMC yield during 1977

Design	Source	df	Germination		No. of millable canes (NMC)		Cane Yield	
			M.S.S.	F	M.S.S.	F	M.S.S.	F
AUGMENTED R.B.D.	BLOCKS	13	192	1.93	239	1.71	140.7091	1.95
	CHECKS	2	538	5.41*	1074	7.67**	680.9090	9.45**
	ERROR	26	99		140		72.0855	
S.E. of difference between a check & a genotype			12		14		10.15	
	L.S.D.		24		29		29.91	
	C.V.		11.38		10.16		14.78	
RECTANGULAR LATTICE	REPLICATIONS	1	0.0055		627		435.7733	
	VARIETIES	89	301.91		954		360.1213	
	BLOCKS (Adj)	18	168.89	1.45	937	28.39**	793.8369	14.01**
	INTRA-BLOCK ERROR	71	116.57		33		−56.6550	
	ERROR-R.B.D.	89	127.1517		216		115.3546	
Effective error variance			123.98		39.93		−59.91	
S.E. of difference between a check & a genotype			11		6		7.74	
	L.S.D.		22		12		15.43	(21.42)
	C.V.		11.49		5.14		12.60	(17.48)

** Significant at 5% level*

*** Significant at 1% level*

Figures in parenthesis are from R.B.D. analysis.

TABLE 2. Analysis of variance for Augmented R.B.D. and Rectangular Lattice Designs for germination, NMC and Yield during 1978

Design	Source	df	Germination		No. of millable canes (NMC)		Cane Yield	
			M.S.S.	F	M.S.S.	F	M.S.S.	F
AUGMENTED R.B.D.	BLOCKS	13						
	CHECKS	3	427	< 1	318	< 1	103.96	1.18
	ERROR	39	578	< 1	2597	5.85**	435.51	4.93**
S.E. of difference between a check & a genotype			589		444		88.34	
L.S.D.			28		24		10.88	
C.V.			57		49		21.98	
RECTANGULAR LATTICE	REPLICATIONS	1	24.84		16.45		23.12	
	VARIETIES	89	653.60		9.00		326.70	
	BLOCKS (adj)	89	361.08		1571.53		313.63	
	INTRA-BLOCK	18	172.77	1.58	232.17	1.93*	66.13	1.18
	ERROR	71	109.02		120.41		55.87	
Effective error variance					143.01		57.94	
S.E. of difference between a check & a genotype			121.92		132.44		57.64	
L.S.D.			117.33		12		7.59	
C.V.			11		23		15.13	
	ERROR (RBD)	89	22		8.34		16.29	
			10.56					

* Significant at 5% level

** Significant at 1% level

TABLE 3. Analysis of variance for Augmented R.B.D. and Cubic Lattice Designs for germination, number of tillers and NMC during 1978-79

Design	Source	df	Germination		No. of tillers		NMC	
			M.S.S.	F	M.S.S.	F	M.S.S.	F
AUGMENTED R.C.B.D.	BLOCKS	4	14.50		78.00	1.86	107.25	4.28
	CHECKS	5	21.80	1	349.20	8.30**	456.80	18.28**
	ERROR	20	35.10		42.30		25.00	
S.E. of difference between a check and new variety			—		7.48		5.77	
	L.S.D.		—		15.63		11.89	
	C.V.		27.65		19.09		15.51	
CUBIC LATTICE	REPLICATIONS	2	12.50		551.27		110.73	
	VARIETIES	215	44.10		188.53		217.26	
	BLOCK (adj)	105	14.84	1.16	46.09	1.14	37.07	1.33
	INTRA-BLOCK ERROR	325	12.75		40.54		27.75	
	R.B.D. ERROR	430	13.26		41.89		30.02	
			13.20		41.75		29.52	
Effective error variance			2.96		5.27		4.44	
S.E. of difference between a check and a new variety			5.80		10.32		8.70	
	L.S.D.		17.59		17.30		15.87	
	C.V.							

** *Significant at 1% level*

TABLE 4. Analysis of variance for Augmented R.B.D. and Cubic Lattice Designs for germination, number of tillers and NMC during 1978-79

Design	Source	DF	Germination		No. of tillers		NMC	
			M.S.S.	F	M.S.S.	F	M.S.S.	F
AUGMENTED	BLOCKS	5	28.00	1.65	29.00	1.10	31.38	1.26
R.B.D.	CHECKS	5	11.60	< 1	288.80	10.91**	260.78	10.94**
	ERROR	25	17.44		26.48		24.83	
S.E. of difference between a check and a variety			—		5.82		5.69	
	L.S.D.		—		11.99		11.72	
	C.V.		18.97		12.34		12.70	
CUBIC LATTICE	REPLICATIONS	2	10.00		3312.42		2648.30	
	VARIETIES	215	24.66		167.64		150.23	
	BLOCKS (adj)	105	13.94	1.20	34.47	1.32	34.06	1.17
	INTRA-BLOCK ERROR	325	11.64		26.07		28.96	
	R.B.D. ERROR	430	12.20		28.12		30.21	
Effective error variance			12.12		26.94		30.04	
S.E. of difference between a check and a variety			2.84		4.24		4.49	
	L.S.D.		5.57		8.31		8.80	
	C.V.		15.56		13.07		14.63	

** *Significant at 1% level*

efficients of variations were uniformly higher than in lattice design except for a marginal difference in germination data in the first location with the first season data analysis. Similarly, the standard errors and least significant differences were also higher in the augmented design analysis compared to lattice. However, this difference was not high with the second season data analysis. For the economic attributes of number of millable canes and cane yield, significant differences among checks were obtained in both locations for augmented design. As the block (adjusted) sum of squares for yield data in lattice design in the first location during 1977 was of a very high order leading to a highly negative value for intra-block error S.S., an ordinary R.B.D. analysis was also carried out and the L.S.D. and C.V. so obtained are indicated in parenthesis in Table 1.

Though the analysis was carried out for all the three characters in both the designs and locations, selection was based on genotypes exhibiting superior performance than the checks in respect of the most important economic characters viz., number of tillers, number of millable canes and cane yield per plot. The number of genotypes selected on the basis of their superiority over the checks in each design and location as well as those selected on the basis of common superiority in both the designs are furnished in Tables 5 and 6.

TABLE 5. Number of genotypes selected in the different designs (1977 - 78)

LOCATION	Total genotypes evaluated	Common in both designs		Only in Augmented		Only in Lattice	
		NMC	Yield	NMC	Yield	NMC	Yield
I	83	10	7	8 (18)	6 (13)	5 (15)	–2* (7)
II	86	11	14	20 (31)	2 (16)	6 (17)	9 (23)

** by R.B.D. analysis*
Figures in brackets indicate total number superior to standards

The total number of genotypes selected on the basis of number of millable canes was more in augmented design than in rectangular lattice in both locations during 1977. For yield, a higher number of genotypes revealed their superiority in the first location in augmented design, but in the second location involving a different set of genotypes, the trend of results was also different. With the data of 1978 season, more or less same number of genotypes came up for selection in location I from NMC analysis. A very high number of genotypes got selected only in lattice design for both the characters in location II. However, in both locations and in both designs during both two seasons, the top 10% superior genotypes were selected.

TABLE 6. Number of genotypes selected in the two different designs during 1978-79

LOCATION	Total genotypes evaluated	Common in both designs		Only in Augmented		Only in Lattice	
		Tillers	NMC	Tillers	NMC	Tillers	NMC
I	210	14	23	20 (34)	16 (39)	8 (22)	14 (37)
II	210	30	19	19 (49)	9 (28)	30 (60)	43 (62)

Figures in brackets indicate total number superior to checks

DISCUSSION

The comparative utility of the augmented designs vis-a-vis lattice designs for evaluation of a large number of genotypes in the early stages of breeding trials in sugarcane has to be assessed from the criteria of their relative statistical efficiency, selection of the superior genotypes without missing the elite ones and practical expediency involving other limiting factors as planting material, land, time and funds.

Basing from the statistical aspect, the lattice design appeared to be more efficient than the augmented R.B.D. because it recorded lesser error variance and lower C.V. in both the locations making it possible to obtain precise estimates for comparison between genotypes in relation to check varieties. However, the error variances did not show much difference in the 1978 trial which may presumably be due to smaller plot size. Though the augmented design also provided for elimination of block effects in respect of each genotype, there seemed to be no possibility of further reduction of error variance. The efficiency of lattice design is much more in this respect and its utility in plant breeding trials has been well established (Yates[4]). In the augmented design, a higher L.S.D. is recorded and at the same time, a higher C.V. has also been obtained.

The number of genotypes selected in the two designs varied considerably in respect of the two characters, viz., the number of millable canes and plot yield during 1977 and NMC and number of tillers during 1978 but the fact to be considered is that in both the lay-outs, the same top 10% elite genotypes got selected. The variation in the number of genotypes selected is attributable to the complex nature of the genotype and environmental interaction, especially for quantitative attributes affecting the yield. However, marginal deficiencies in any design have to be ignored for the sake of many favorable points in adopting an alternative, convenient and effective design. In the advanced clonal evaluation in sugarcane crop, about 80 to 100 genotypes are tested,

whereas in the earlier stage of pre-final clonal trial, as many as 500-600 genotypes are being evaluated every year at Sugarcane Breeding Institute. In such situations, the check-plot method may not be efficient and the adoption of lattice design with minimum of two replications also may not be practicable on account of limitations of planting material. Hence, the augmented design can be conveniently employed at these stages of selection trials. This augmented randomized block design has the following special features of i) elimination of the need for replicates, ii) removal of block effects for each genotype like any other incomplete block design, iii) providing a valid estimate of the error variance for genotypes and comparisons among genotypes, standards and between members of the two groups and iv) reducing the experimental area. The present results indicate that no serious disadvantage is likely to be encountered in employing the augmented design for selection experiments in crops like sugarcane as compared to lattice designs for the selection of superior clones in the initial stages of breeding trials.

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LA ADECUACION DE LOS DISEÑOS AUMENTADOS AL MEJORAMIENTO GENETICO

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RESUMEN

Se estudió en forma comparativa la eficacia del diseño aumentado y del "lattice" para la identificacion de clones superiores de caña de azucar en los programas de mejoramiento. La investigacion se baso en dos grupos diferentes de genotipos, cada uno de ellos en dos localidades y años diferentes en el Instituto de Mejoramiento de Caña de Azucar de Coimbatore.

Se observó que los 10 mejores genotipos a seleccioñar sobre

la base de cualquiera de estos diseños son practicamente los mismos. El diseño aumentado, si bien no igual al "lattice", trata de lograr la mayor eficiencia estadistica posible en su disposicion y parece ser un diseño util para los fitomejoradores de caña de azucar que deben evaluar un elevado numero de genotipos en la forma mas precisa posible, a pesar de algunas limitaciones como ser la disponibilidad de semilla. Tambien puede ser considerada una tecnica muy util desdel punto de vista economico.

PRELIMINARY STUDIES ON THE MODE OF INHERITANCE OF RESISTANCE TO WIND-BREAKAGE IN SUGARCANE

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ABSTRACT

A total of 397 sugarcane clones of the first vegetative propagation derived from 30 cross combinations were tested against the typhoon Thelma in 1977.

Resistance is partially dominant over susceptible responses in sugarcane against the wind-breakage which may involve several genes. The results indicated that the degree of resistance to wind-breakage was correlated with the resistance or susceptibility of parent varieties. Percentages of sugarcane clones showing responses were: 66.2% in resistant X resistant (R X R), 56.7% in resistant X susceptible (R X S), 46.5% in susceptible X resistant (S X R) and 28.9% in susceptible X susceptible (S X S) combinations. The average percentage of wind-breakage of the sugarcane clones were 19.95% in R X R, 24.95% in R X S, 34.3% in S X R and 37.26% in S X S combinations.

The regression analysis on the percentage of wind-breakage between parental varieties and their progenies indicated that the trait of resistance to wind-breakage was mainly determined by the maternal effect. The parental effect, however, was not important. It was proposed that both the genes in nucleus and also certain genetic factors in the maternal cytoplasm are involved in the genetic control of wind-breakage resistance of sugarcane.

The genotypes of the wind-breakage resistance of the parental varieties are, more or less, heterozygous in sugarcane.

INTRODUCTION

According to reports of Chao[1] and Hsieh[2], a wind velocity faster than 15 m/sec might split the cane leaves and interrupt photosynthesis while a wind velocity up to 20 m/sec could break the cane stalks, resulting in pathogen and insect invasion of cane stem, thus lowering the quality of millable stalk. In Taiwan, the variety F134 was rapidly eliminated during 1950-54, due to susceptibility to wind-breakage and the release of new widely adaptable variety

NCo310. Therefore, the cheapest way to solve the problem of wind damage on sugarcane is the selection and breeding of resistant varieties.

On July 25, 1977, typhoon Thelma attacked the southern part of Taiwan causing heavy damage to sugarcane in the Pingtung area. Most of cane leaves were split and the stalks were not only broken but also seriously lodged. During that period the crossing nursery was inspected and a significant difference in the percentage of wind-breakage among different varieties was observed. To clarify the different genetic mode of combinations resistant to wind-breakage, an investigation was conducted to offer sugarcane breeders a reference before designating the cross combinations. Genetic mode of resistance to wind-breakage has thus far never been reported in sugarcane.

MATERIALS AND METHODS

On July 25, 1977, typhoon Thelma attacked the Pingtung area with a maximum velocity of 32.7 m/sec and caused heavy damage to the sugarcane. After it was over, the percentages of wind-breakage on parental varieties were investigated in the spring planted crossing nursery at the Wantan Sugarcane Breeding Station. Among them, 12 varieties were selected as wind resistant (R) including 6 highly wind resistant (HR), 2 moderate (M) and 10 susceptible (S) including 5 highly wind susceptible (HS) (Table 1). Among these 24 varieties, 30 cross combinations were picked out for growing. We found out that 5 combinations were resistant for both parents (R X R). 5 combinations were female parent resistant and make parent susceptible (R X S), 7 combinations were male parent resistant and female parent susceptible (S X R), 6 combinations were susceptible for both parents (S X S). Thus, these constitute four main types of cross combination. The numbers of the cross combination for the remaining 9 different combination types are listed in Table 5. The tassel used for pollination was randomly picked up among tasseling plants of a parental variety.

The selected seedlings of the first vegetative propagation were planted each clone having one single 6-meter row. The percentages of wind-breakage were investigated. Fifteen to 18 clones were randomly surveyed from each cross combination. All clones were surveyed if the samples were less than 15 clones per cross combination. The grade of wind-breakage of the parental varieties is listed in Tables 1 and 2.

RESULTS

Comparison of the sugarcane clones, response to wind resistance derived from the different cross combinations are listed in Tables 3 and 4. Variety F162 is one of the highly susceptible varieties (Table 1). When used as a pollen parent crossed with wind resistant, seed parent (R X S), an average of 57.1% wind-resistance progenies were obtained. But when F162 was used as a seed parent crossed with wind-resistant pollen parent, 45.8% wind-resistant progenies were produced. Furthermore, when F162 was used as male or female parent

crossed with wind susceptible clones, the percentage of wind resistant progeny decreased drastically. The variety 63-632 a susceptible type (S) when used as a seed parent and crossed with wind-resistant pollen parent (R). 55.6%-57.9% of the R type progenies were produced. When it was crossed with M type (63-632 X F152) or S type (63-632 X F162) only 15.4% and 18.8% S type progeny were obtained, respectively.

TABLE 1. Classification of the response of the parental varieties to tyhpoon Thelma

Categories		Numerical grading	Wind-breakage %	Parental varieties
Resistant (R)	Highly Resistant (HR)	0, 1	0 3	F141, F153, F158, F160, F173, 58-2400
	Resistant (R)	2, 3, 4	4 11	F166, F168, F169, F171, F176, PT43-52
Moderate (M)	Moderate (M)	5, 6	12 31	F152, F178
Susceptible (S)	Susceptible (S)	7, 8	32 80	F156, F164, 57-296 63-632, 68-276
	Highly Susceptible (HS)	9	81 100	F162, 51-213, 54-2011 59-2033, 64-1534

TABLE 2. Percentages of wind-breakage of the parental varieties to typhoon Thelma

Parental varieties	% of wind-breakage	Parental varieties	% of wind-breakage
F141	3.0	F173	2.4
F152	12.5	F176	11.0
F153	0.2	F178	30.0
F156	63.0	PT43-52	10.0
F158	0	51-213	100.0
F160	3.1	54-2011	80.0
F162	84.4	57-296	40.0
F164	78.3	58-2400	3.0
F166	7.5	59-2033	95.0
F168	6.0	63-632	35.0
F169	10.0	64-1534	90.0
F171	6.0	68-276	40.0

Apparently, wind susceptible clones should be crossed with R type and we should avoid using them as a seed parent. The M type, like F152,

TABLE 3. Comparison of the response of the sugarcane derived from the same male parent but different female parent among combination groups in the field to typhoon Thelma.

Combinations	Parentages	No. of clones investigated	Response of clones R %	M %	S %	% wind-breakage Range	Mean X±SE	Coefficient of variation of wind-breakage %
HR X HR	F158 X F141	15	73.3	20.0	6.7	6-45	14.1±10.9	77.2
HS X HR	F162 X F141	12	33.3	0	66.7	9-70	40.7±25.1	61.7
HR X M	F160 X F152	14	78.6	14.3	7.1	6-45	13.3±11.4	86.1
R X M	F171 X F152	16	87.4	6.3	6.3	3-70	12.4±16.7	134.9
M X M	F178 X F152	15	26.7	13.3	60.0	9-90	44.7±28.7	64.2
S X M	63-632 X F152	16	18.8	25.0	56.2	9-70	40.9±24.9	61.0
HS X M	F162 X F152	18	22.2	16.7	61.1	9-70	40.3±22.4	55.5
S X HR	63-632 X F160	19	57.9	10.5	31.6	9-90	27.9±26.6	95.5
S X HR	F156 X F160	7	57.1	14.3	28.6	6-90	31.0±34.2	110.4
HR X HS	F158 X F162	8	50.0	25.0	25.0	6-45	23.6±16.3	69.1
R X HS	F169 X F162	9	44.5	11.0	44.5	6-70	31.9±26.1	81.9
R X HS	F176 X F162	13	76.9	7.7	15.5	6-70	17.1±19.0	111.1
S X HS	57-296 X F162	16	43.8	18.8	37.4	6-70	29.9±25.5	85.0
S X HS	63-632 X F162	13	15.4	53.8	30.8	9-70	29.9±19.2	64.2
HS X HS	59-2033 X F162	7	0	28.6	71.4	20-70	50.7±22.8	45.0
M X R	F178 X F171	7	57.1	14.3	28.6	6-70	31.9±34.5	108.2
S X R	63-632 X F171	18	55.6	0	44.4	6-90	33.9±31.7	93.5
HS X R	59-2033 X F171	17	29.4	17.6	53.0	9-90	40.2±28.9	71.8
HR X HS	F160 X 54-2011	15	46.7	20.0	33.3	3-70	27.9±25.3	90.6
M X HS	F152 X 54-2011	9	0	22.2	77.8	20-90	56.1±24.1	42.2

Note: HR: Highly resistant to wind-breakage
R: Resistant to wind-breakage
M: Moderate
S: Susceptible to wind-breakage
HS: Highly susceptible to wind-breakage

when used as a male parent crossed with R type (F160 X F152, F171 X F152) 78.6%-87.4% of R type progenies were obtained, while when crossed with M or S type, the R type progeny were less than 27% (Table 3 and Table 4). So we avoid crossing the M type with M or S type.

Through the typhoon Thelma test, we found that cultivar F160 belonged to a highly resistant variety. No matter whether it was used as a male or female

TABLE 4. Comparison of the response of the sugarcane clones derived from the same female parent but different male parent among combination groups in the field to typhoon Thelma.

Combinations	Parentages	No. of clones invest-igated	Response of clones			% wind-breakage		Coefficient of variation
			R %	M %	S %	Range	Mean X+SE	of wind-breakage %
HR X HR	F158 X F141	15	73.3	20.0	6.7	6-45	14.1±10.9	77.2
HR X HS	F158 X F162	8	50.0	25.0	25.0	6-45	23.6±16.3	69.1
HR X HR	F160 X F173	9	55.6	11.1	33.3	6-70	24.7±23.0	93.3
HR X HR	F160 X F166	16	75.0	12.5	12.5	3-70	15.7±17.5	111.6
HR X R	F160 X F168	12	50.0	16.7	33.3	6-90	30.5±31.3	102.7
HR X M	F160 X F152	14	78.6	14.3	7.1	6-45	13.3±11.4	86.1
HR X HS	F160 X 54-2011	15	46.7	20.0	33.3	3-70	27.9±25.3	90.6
HS X HR	F162 X 58-2400	12	58.3	25.0	16.7	3-70	19.5±19.3	99.0
HS X HR	F162 X F141	12	33.3	0	66.7	9-70	40.7±25.1	61.7
HS X M	F162 X F152	18	22.2	16.7	61.1	9-70	40.3±22.4	55.5
S X R	F164 X PT43-52	16	37.5	0	62.5	6-90	47.0±35.2	75.0
S X S	F164 X 68-276	8	50.0	16.7	50.0	6-70	33.1±27.8	83.8
R X HR	F176 X F153	13	69.2	23.1	7.7	6-45	15.1±10.5	73.6
R X HS	F176 X F162	13	76.9	7.7	15.4	6-70	17.1±19.0	111.2
M X R	F178 X F171	7	57.1	14.3	28.6	6-70	31.9±34.5	108.2
M X M	F178 X F152	15	26.7	13.1	60.0	9-90	44.7±28.7	64.2
HS X R	59-2033 X F171	17	29.4	17.6	53.0	9-90	40.2±28.9	71.8
HS X HS	59-2033 X F162	7	0	28.6	71.4	20-70	50.7±22.8	45.0
S X HR	63-632 X F160	19	57.9	10.5	31.6	9-90	27.9±26.6	95.5
S X R	63-632 X F171	18	55.6	0	44.4	6-90	33.9±31.7	93.5
S X M	63-632 X F152	16	18.8	25.0	56.2	9-70	40.9±24.9	61.0
S X HS	63-632 X F162	13	15.4	53.8	30.8	9-70	29.9±19.2	64.2
HS X HS	64-1534 X F162	16	37.5	25.0	37.5	9-90	30.8±24.1	78.3
HS X HS	64-1534 X 51-213	16	18.8	12.5	68.7	9-90	49.0±27.3	55.6
HR X HS	F173 X 64-1534	15	60.0	20.0	20.0	9-90	24.2±25.1	103.9

parent crossed with different types of parent, more than 50% of R type progeny was obtained. The best combination was to use F160 for female crossed with F152 or F166 for male, then more R type progeny would be acquired. Cultivar F176 was a good female parent. When it was used for female crossed with R type male F153, or S type F162, the R type progeny were around 69.2%-76.9%.

Both varieties of 59-2033 and 64-1534 were of the highly wind susceptible type. The combinations of 59-2033 X F171 (HS X R) and 59-2033 X F162 (HS X HS) only produced 29.4% and 0% of the type progenies, respectively In the combinations of 64-1534 X F162 (HS X HS) and 64-1534 X 51-213 (HS X HS) combinations, only 37.5% and 18.8% R type progenies of genetic segregants were obtained, respectively. However, in the combination of F173 X 64-1534 (HR X HS), the R offspring was increased up to 60%. This indicated that S type variety should not be used as female parent.

The response of the four major combinations to wind-breakage is summarized as follows. (Table 5).

(1) In 5 combinations of R X R, there were 66.2% R type and 16.9% S type among 65 progeny clones.
(2) In 5 combinations of R X S, there were 56.7% R type and 26.6% S type among 60 progeney clones.
(3) In 7 combinations of S X R, there were 46.5% R type and 44.6% S type among 101 progeny clones.
(4) In 6 combinations of S X S, there were 28.9% R type and 47.4% S type among 76 progeny clones.

TABLE 5. Summarized results of the response of the sugarcane clones of each combination group in the field to typhoon Thelma

Combination	No. of crosses	No. of clones investigated	Response of clones			% of wind-break		Coefficient of variation of wind-breakage percentage %
			R %	M %	S %	Range	Mean X+SE	
R X R	5	65	66.2	16.9	16.9	3-90	19.85±7.39	91.68
R X S	5	60	56.7	16.7	26.6	3-90	24.95±5.51	91.34
R X M	2	30	83.3	10.0	6.7	3-70	12.84±0.64	109.60
S X M	2	34	20.6	20.6	58.8	9-70	40.60±0.39	58.20
M X R	1	7	57.1	14.3	28.6	6-90	31.86	108.20
M X M	1	15	26.7	13.3	60.0	9-90	44.67	64.20
M X S	1	9	0	22.2	77.8	20-90	57.22	42.20
S X R	7	101	46.5	8.9	44.6	3-90	34.30±9.20	86.70
S X S	6	76	28.9	23.7	47.4	6-90	37.26±9.85	68.65
Total	30	397	46.8	15.9	37.3	3-90		

Using R type as a female parent instead of S type in R and S combination, we can increase 10.2% R type progeny and reduce 18% of S type progeny.

The frequency of offspring resistant to wind-breakage decreased following the sequences of (M X R) (M X M) (M X S) or (R X M) (M X M) (S X M);

on the contrary the frequency of progeny susceptible showed just the opposite direction.

From Table 5 it can be seen that the average percentage of wind-breakage progeny is 19.85% in combination of R X R, and 12.84% in combination of R X M. The other combinations were all higher than 31%. The wind-resistant progenies were obtained from each of the 30 cross combinations, except two combinations of 59-2033 X F162 (HS X HS) and F152 X 54-2-11 (M X HS).

As shown in Tables 3-5, it may be observed that the range of variation of sugarcane resistance to wind-breakage has a significant genetic segregation. From the results of three combinations of HS X HS (59-2033 X F162, 64-1534 X F162 and 64-1534 X 51-213), the R type progenies were 0, 18.5% and 37.5%, respectively. This implies that the trait of sugarcane resistant to wind-breakage may be controlled by several pairs of partially dominant genes or polygenes which constitute the quantitative inheritance. This proposal is further supported by the results that the frequencies of the R progenies were 66.2%, 56.7%, 46.5% and 28.9% in R X R, R X S, S X R and S X S combinations, respectively.

A random survey was made on the different locations of the check variety F156 for its wind-breakage percentage. Of samples investigated in 17 different spots, 59% has a wind-breakage of around 4-11%, 35.3% has a wind-breakage of around 12-13%, and 58.8% has a wind-breakage around 32-100%. Therefore, the velocity of the wind is not evenly but is randomly distributed throughout the field. We can not exclude the possibility of the higher wind-breakage percentage for certain sugarcane clones located in the strong wind area. Based on the results derived from this study, it can be seen that one should first concentrate on selecting the proper parentage before designating the cross combinations. From the viewpoint of wind-breakage-resistant breeding, female and male parents which are both resistant to wind-break will be the first choice. When only one of the parents is a wind resistant type, the better alternative, if possible, is to use it as a female parent.

There are certain wind susceptible materials which we should not abandon if they have other good characteristics, because through their derived offspring, we can still find wind-resistant individuals. In our investigations there were 28.9% of wind-resistant offspring from the S X S cross combinations.

DISCUSSION

There was no offspring derived from the self-fed parental varieties on this study. It is difficult to ascertain how many pairs of genes were involved in wind-breakage resistance in sugarcane. And we are not sure whether the genotype of the parental varieties is either homozygous or heterozygous. However, the degree of homozygosity of the parental varieties can still be estimated by evaluation of the value of the coefficient of variation (CV) from a given parental combination. The higher the degree of the homozygosity of the parental

genotype resistant or susceptible to wind-breakage, the smaller the CV of the progeny. And this results in a genetic variation of wind-breakage among progeny. On the other hand, a higher CV implies that there are more possibilities for the genotypes of the parents to exist as heterozygous since the wind genetic genotype resistant or susceptible to wind-breakage, the smaller the CV of the progeny. And this result in a genetic variation of wind-breakage among progeny. On the other hand, a higher CV implies that there are more possibilities for the genotypes of the parents to exist as heterozygous since the wide genetic variation of wind-breakage among the progeny is due to transgressive segragation.

There are 9 combination types in 30 cross combinations among our survey, the R X M and S X M with 2 cross combinations each, and M X R, M X M and M X S with only 1 combination each. Because the number of combinations for each type was not enough, and the number of clones of progeny of each cross combination being investigated was only 15-18, therefore, the reaction of these five types of cross combination were listed only four reference. The 4 types of combination R X R, R X S, S X R and S X S constitute 32 cross combinations and the CV of the percentage of wind-breakage of the offspring ranged from 45.0 to 111.6. We may assume that the genotypes of wind-breakage-resistance of the parent would exist, more or less, as heterozygosity.

TABLE 6. ANOVA of coefficient of variation of the percentage on wind-breakage among combination groups of R X R, R X S, S X R and S X S

Source of variation	Degrees of freedom	Mean square	F
Wind resistance combinations	3	676.31	2.40*
Error	19	281.78	
Total	22		

**Significant at 1% level*

The analysis of variance of the coefficient of variation (CV) for the 4 major kinds of combinations (Table 6) is significant at 10% level. Examination of the CV of the percentage of wind-breakage of the offspring showed that there were 91.68% in R X R, 91.34% in R X S, 86.70% in R X S, 86.70 in S X R and 86.65% in S X S. We found out that the CV of S X S was far lower than the others. However, the average wind-breakage of S X S combination is 37.26% which is the highest of the 4 major combinations. This implies that in the S X S combinations the genotypes of parents consist of more recessive wind-susceptible genes which have more opportunities of existing as homozygote.

Among S X S combinations, the progeny of 59-2033 X F162 and 64-1534 X 51-213 have smaller CV but higher percentage of wind-breakage. Therefore, the genotypes of wind-breakage resistance of these four parental varieties will have a great degree of homozygosity. Their parental varieties were classified

as of a highly wind-susceptible category (Table 1). There are 0.50% with an average of 28.9% wind-resistant progeny of genetic segragation among the 6 S X S combinations (Table 5). However, whether the wind-resistant progeny is a result of the recombination of the genes from different germplasm of the separate wind-susceptible parent or is a result of the intercation of genes derived from both parents is still not known yet. The percentage of wind-breakage of the offspring from the R X R, R X S and S X R combinations showed that the average coefficient of variance is pretty close. However, they were all higher than S X S combination. This indicated that the degree of heterozygosity or genetic diversity in terms of wind-resistance is higher in the parental varieties of R X R, R X S and S X R combinations than that of S X S combination.

In the R X R combination, the average wind-breakage of the F158 X F141 is 14.13%, and CV is 77.2%. The offspring includes 73.3% R type and 6.7% S type individuals (Table 3). The average wind-breakage in F176 X F153 (R X R) is 15.1%, and CV is 73.6%, and the offspring includes 73.6% R type and 7.7% S type individuals (Table 4). Thus, we can infer that these four parents have more partially dominant wind-resistant genes than recessive wind-susceptible genes. Therefore, a higher degree of homozygosity in genotypes of wind-breakage resistance for the above four parents than the other parents of R X R combination will be expected. Among these four parental varieties, F141, F153 and F158 belong to highly wind-resistant varieties (Table 1).

The coefficient of variation (CV) of the percentage of wind-breakage from progeny is pretty close among the cross combinations of R X S and S X R. Among five cross combinations of R X S, the range of CV was 69.1% to 103.9% with an average 91.34%. And the CV of the 3 combinations is higher than 84.09%, which is the average CV of the 23 combinations. Among seven combinations from S X R, the range of CV was 61.7% to 110.4% with the average 86.7%. There are 4 combinations with the higher CV value than the average CV 84.09%. The results indicated that among these combinations, except those in which the germplasm of the parent is from a different source, most parents have the genotypes of wind-resistance existing as heterozygous.

The progeny from the combination F162 X F141 (S X R) has an average wind-breakage of 40.7% and CV of 61.7%. There are 33.3% of wind-resistant segregation offspring and 66.7% of wind-susceptible offspring. This implies that more wind-susceptible recessive genes constitute the wind-susceptible genotype of variety F162. The reverse is true for the male parent F141. The degree of homozygosity in the genotype of wind-breakage may be pretty high for both parents. As a consequence, the genotypes of most progeny are heterozygous with a wind-breakage of moderate resistance. This results in a narrow variation in terms of the percentage of wind-breakage and is confirmed by a small CV of 61.7%. From Table 1 we can see that the female parent F162 is a highly wind-susceptible variety but the male parent F141 is a wind-resistance variety.

A similar case was found in the R X S combination such as F158 X F162, The CV value of the progeny is only 69.1%. The parent F158 was highly wind-resistant and F162 was highly wind-susceptible (Table 1).

On the basis of these results, we may infer that the bigger CV value of the wind-breakage percentage in the progeny means their parents have more opportunities of heterozygous genotype for wind-resistance. So that the recombination of the wind-resistant genes or the wind-susceptible genes in the genotypes of the progeny will be increased. This results in the broadening of the genetic variation in terms of wind-breakage resistance among offspring.

Although the CV's of the wind-breakage percentage of the offspring was pretty close on the R X S and S X R combinations, the former progeny has 24.95% of wind-breakage while the latter has 34.3%. This apparently shows that the influence of the female parent is much greater than that of the male parent. For example, the average wind-breakage of the offspring is only 13.3% in F160 X F152 (R X M) and 12.4% in F161 X F152 (R X M). There are 78.6% and 87.4% wind-breakage resistant progenies on these combinations, respectively. However, the average wind-breakage of the offspring was increased up to 40.9% in 63-632 X F152 (S X M) and 40.3% in F162 X F152 (S X M). Only 18.8% and 22.2% of wind-breakage resistant progenies were obtained in these two combinations (Table 3). This indicates the tendency of maternal effect (cytoplasmic inheritance) of wind-breakage resistance in sugarcane.

TABLE 7. ANOVA of the offspring-maternal regression

Source of variation	Degree of freedom	Mean square	F
Regression	1	11,671.96	13.13*
Residue	28	888.99	
Total	29		

Y = 14.62 + 1.69X

*Note: ** Significant at 1% level*

After regression of the wind-breakage percentage of the offspring on the female parents among 30 combinations, we obtain a regression equation $Y = -14.62 + 1.69X$. It is significant at 1% level through a regression analysis of variance. The results again indicate that the transmission of resistance of wind-breakage is mainly through the female parents. This is because the value of regression coefficient can serve as an indirect index of the heritability. The correlation coefficient of offspring and their female parents of the wind-breakage percentage $r = 0.56$ is also significant at 1% level.

The regression equation of the wind-breakage percentage between offspring and their male parents is $Y = 15.56 + 0.69X$. Through the analysis of variance (Table 8) there was no significant difference, neither was their correlation

TABLE 8. ANOVA of the offspring-paternal regression

Source of variation	Degree of freedom	Mean square	F
Regression	1	1,972.00	1.35^{NS}
Residue	28	1,464.31	
Total	29		

Y = 15.56 + 0.69X
Note: ^{NS} *Not significant*

coefficient r = 0.21 significant. This means that the transmission of wind-breakage resistance comes very little through the male parent.

The maternal effect of resistance to wind-breakage can be calculated as follows:

$$\left\{[(R \times R) + (R \times S)] - [(S \times R) + (S \times S)]\right\}/2 = \left\{[(19.85 + 24.95) - (34.30 + 37.26)]\right\}/2 = -13.38 \text{ (Table 4).}$$

This indicates that there was a 13.38% decrease of wind-breakage when we use R type instead of S type as female parents in crosses.

The paternal effect of wind-breakage resistance also can be calculated as follows:

$$\left\{[(R \times R) + (S \times R)] - [(R \times S) + (S \times S)]\right\}/2 = -4.03$$

This reveals that there was only 4.03% of wind-breakage decrease when we use R type instead of S type as male parent.

Consequently, we believe that both the genes in nucleus and also certain genetic factors in maternal cytoplasm are involved in the genetic control of wind-breakage resistance of sugarcane. The genotypes of parental varieties showing wind-breakage resistance are, more or less, heterozygous in sugarcane.

According to the reports of Chao[1] and Hsieh[2], the factors which influence sugarcane wind-breakage include the toughness of the stem, size of shoot, fiber content and rind hardness of the internodes. Therefore, wind-breakage resistance is not determined by a single factor but is a combined effect of several factors. As a consequence, any gene which control the performance of the above factors will finally be involved in the resistance of the wind-breakage of sugarcane.

There is no way to avoid the threat of typhoons in Taiwan, thus, the improvement of sugarcane has been concentrated on the breeding of hard cane. Loh *et al*[3,4] started the basic research on the rind hardness of sugarcane

internodes in 1949. They graded the varieties according to the rind hardness of the stalk. Their co-worker Sung Lee adopted the system for reference before designating the cross combinations. The hard sugarcane with the lowest wind-breakage among commercial varieties in this island was PT43-52 in 1952. Because of its high susceptibility to downy mildew, PT43-52 was no longer a commercial variety. However, it has become one of the most important parental canes in Taiwan for its good agronomic and wind-resistance traits.

The NCo310 became the main commercial variety from 1953 until it was replaced by F146 in 1966[5]. F146 was derived from NCo310 X PT43-52. Through more than one decade of cultivation in Taiwan, NCo310 has been proved as a wind-resistant variety. Both NCo310 and PT43-52 were very important parentage in Taiwan.

Of the 24 parental varieties investigated, the varieties F141 (HR), F152 (M), F156 (S), F158 (HR), 64-1534 (HS) and 68-276 (S) contain one-half to one-greater of the PT43-52 pedigree. F153 (HR) has half pedigree of NCo310. The varieties F160 (HR), F166 (R), F168 (R), F169 (R), F171 (R), F176 (R), 58-2400 (HR) and 63-632 (S) hold one-half to one-quarter of the pedigree from both PT43-52 and NCo310. The varieties F162 (HS), F173 (HR) and F178 (M) have the pedigree of F134. PT43-52 and NCo310. F164 (S) has the one-quarter pedigree of F134 and NCo310. The rer. aining parentages such as 51-213 (HS), 54-2011 (HS), 57-296 (S) and 59-2033 (HS) have no genetic relationship with PT43-52 or NCo310. Apparently, most wind-resistant parental varieties used in this study had the pedigree of either PT43-52 or NCo310 or both.

Of the 24 parental varieties investigated, 9 varieties had a percentage of wind-breakage lower than PT43-52 which was the most wind-resistant variety in 1952. During the last three decades, sugarcane breeders of this island have put a lot of effort into the breeding and selection of wind-resistant varieties. As a consequence, through recurrent selection, the gene frequency of wind-breakage resistance of the sugarcane varieties has been increased in Taiwan during the last thirty years.

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ESTUDIOS PRELIMINARES SOBRE LA FORMA DE HERENCIA DE LA RESISTENCIA AL DAÑO POR VIENTO EN CAÑA DE AZUCAR

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RESUMEN

Un total de 397 clones de caña de azucar en su primera propagacion vegetativa y derivados de 30 combinaciones parentales, fueron controlados por su comportamiento frente al tifon Thelma en el año 1977.

La resistencia al daño por viento es parcialmente dominante sobre respuestas de susceptibilidad en la caña de azucar. Varios genes o quizas poligenes pueden estar involucrados en esta caracteristica. Los resultados indicaron que el grado de resistencia en los clones esta asociado a la resistencia o susceptibilidad de los padres. Los porcentajes de clones que mostraron resistencia en diferentes tipos de cruzas fueron los siguientes: 66.2% en cruzas de resistente x resistente (RxR), 56.7% en resistente x susceptible (RxS), 46.5 en susceptible x resistente (SxR) y 28.9% en susceptible x susceptibles (SxS). El daño por viento porcentual promedio en los clones fue de 19.95% en RxR, 24.95% en RxS, 34.3% en SxR y 37.26% en SxS.

En analisis de regresion en el porciento de daño por viento entre los padres y sus progenies, indicó que la condicion de resistencia esta determinada principalmente por efecto materno. El efecto paterno no fue importante. Se propone que tanto genes en el nucleo como ciertos factores geneticos en el citoplasma estan involucrados en el control genetico de la resistencia al viento en caña de azucar.

El genotipo de la resistencia al daño por viento en los progenitores es mas o menos heterocigota en caña de azucar.

COPERSUCAR BREEDING PROGRAM

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ABSTRACT

The Copersucar breeding program was started in 1968 with the installation of the Camamu Experiment Station in the State of Bahia for the obtention of cane seeds. The seed germination and the clone selection is carried out in the experiment stations situated in the State of Sao Paulo and under the ecological cultivating conditions of the future SP varieties.

The climatic conditions in Camamu are especially favorable to sugar cane tasseling and seed production. The amount of seeds obtained meets the demands of several research institutes. By means of tasseling control it is possible to coincide the tasseling of a great number of progeny at the same time. The viability of the seeds is increased by specific treatments. The first six SP varieties resistant to smut and mosaic were released in 1978.

INTRODUCTION

The Copersucar breeding program began in 1968 by the enterprise of the "Cooperative Central dos Produtores de Azucar e Alcool" which comprises 74 sugar mills in the center-south region of Brazil. On the coast of the State of Bahia a crossing station has been installed in an area offering more favorable conditions to flowering than were found in the State of Sao Paulo. The Camamu Experiment Station, longitude $39^{o}07'W$, latitude $13^{o}55'S$, is placed in an especially appropriate region to obtain sugarcane seeds for crossing researches.

In 1969 the first tassels were cut. They came from the collection planted the year before. The crossing campaign is held every year in May and June. The seeds obtained are sent to the Copersucar Experiment Stations in the State of Sao Paulo where they are sown. The clones are selected and multiplied in the fields of the cooperated sugar mills.

The Experiment Station Itamogi, in the State of Parana, has been especially installed by Copersucar to test the resistance to the diseases which occur most frequently in the sugarcane regions of the State of Sao Paulo where the future varieties will be planted. These varieties have been given the name SP, registered with the ISSCT.

PURPOSE OF THE BREEDING PROGRAM

The purpose of the program is the production of sugarcane varieties adapted to the edaphological conditions of the regions where the mills affiliated to Copersucar are installed. These are situated in the center-south region of Brazil between the parallels 23° and 15°S. The sugarcane is planted on a strip of land which goes from sea-level (Campos, State of Rio de Janeiro) to 840 m altitude (Sao Carlos, State of Sao Paulo).

When selecting the clones, special importance is given to the following criteria:

- Productivity of the sugarcane aiming at a bigger quantity of sugar produced per area, according to the economical standard established by Hugot[4].
- Ratooning ability under the conditions of low temperature and deficient rainfalls at the time of harvesting.
- No tasseling under the climatic conditions of the center-south region of Brazil.
- Adaptability to low fertility soils (cerrado).
- Ability to mechanical harvesting.

EDAPHOLOGICAL CONDITIONS

The woods which covered the 50 ha of the Camamu Experiment Station were partially cut down for the plantations. The soil of the upper parts of the station where sugarcane is planted is latosol (Typic Haplorthox). Its iron content is low, its aluminum content high and there is low saturation of bases and little natural fertility. The retention of humidity of this argillaceous siliceous soil is low, its porosity high, permitting good development of the sugarcane root system. Due to the use of fertilizers applied at several times during the year and the heavy rainfalls, the sugar cane vegetation is vigorous.

CLIMATIC CONDITIONS FOR THE TASSELING OF SUGAR CANE

After several years of metereological observations it has been possible to gather the information characterizing the climate of the Camamu Experiment Station.

Annual medium maximum temperature	:	28.0°C
Annual medium minimum temperature	:	20.1°C
Monthly maximum temperature: January	:	29.8°C
Monthly minimum temperature: August	:	17.9°C
Annual medium relative humidity	:	78.3%
Annual rainfalls	:	2013.5 mm
Annual number of rainy days	:	275 days
Annual solar illumination	:	2148.3 hours
Annual medium solar radiation	:	425.9 cal/cm^2/day

These characteristics of the Camamu climate correspond in Koeppen's classification to the tropical climate Af. The amplitude of the variations during the year is small and there are no limiting levels to tasseling and the viability of pollen.

At the time of the floral induction in January, February and March the dark period varies from 11 h 14' to 12 h 15'.

ORIGIN OF THE INTRODUCED VARIETIES

In 1969 a research was carried out in order to gather a collection of progeny constituted by all the varieties existing in the experiment stations and private collections of the sugar mills from all parts of Brazil. The purpose of this research was the study of the very often little known genetic potential of these varieties.

During the following years this collection was completed with varieties imported from Canal Point, Hawaii, Argentina and Australia according to J.A. Mangelsdorf's[9] suggestions.

ORGANIZATION OF THE COLLECTIONS

All the varieties are planted in a general collection in which each variety undergoes a rigorous identification control. This collection provide the basic observations about the tasseling of each variety under the climatic conditions of Camamu. It is cut once a year, at the end of June, supplying the seed-pieces for the plantation of the crossing collections. The tassels of the general collection are not used for the crossings.

The crossing collections used for the harvesting of the tassels and which are originated from the general collection, are planted in several sorts of soil and topography. The plantations are arranged in such a manner that they are exposed to sunlight in different ways. Each collection is grouped according to its period of tasseling.

After several years of observation a system of plantation in holes has been adopted. The space is standardized: 2.5 m between the holes and 4 m between the rows. This space permits the maximum exposure to sunlight in relation to the natural potential of Camamu. At the same time it allows the weed control by rotary cut and facilitates the phytosanitary control executed by tractor. It offers as well an easier access at the occasion of the harvesting of the tassels even when the sugar canes are well developed.

The number of the holes planted of each variety goes from 4, 8 to 12, depending on the importance of the progeny for the crossings.

CROSSING CAMPAIGN

The crossings are carried out in May and June. The tassels are cut and maintained alive in an acid solution. The crossings are performed under open rainshelters. In order to prepare the acid solution, the use of pure water from various origins has been tried out. At present rainwater is being used. When there is a lack

of rain, stream-water is used after having suffered an adequate treatment (Miocque[10]) with bentonite and aluminum sulphate. The results were favorable. From the 1103 existing varieties in the collection only 879 varieties have been used as progeny.

During the 1978 crossing campaign, 49.421 kg of seeds were produced. The medium germination rate was of 160 seedlings per gram. The seed production is alloted to the annual Copersucar program of 2 million seedlings, to experiments and to the supplying of seeds to the agreements with the Instituto Agronomico of Campinas, Brazil (5. 5. 871 kg), the Estacion Experimental Agricola of Tucuman, Argentina (0.995 kg), and the Victoria Milling Co, Philippines (0.220 kg).

TASSELING OBSERVATIONS

At the Camamu Experiment Station the conditions for natural tasseling are particularly favorable. The tasseling starts at the end of April and goes till the end of June, but there is a greater intensity between May 10th and 20th, when 60% of the varieties flower. The tasseling rate is 92%.

The tasseling observations are made according to the classification of the tasseling periods of each variety. These records make it possible to establish the program of the crossing to be followed each year. The sex rating is carried out during the crossing campaign. There are 491 male progeny (sex 1-2-3), 184 intermediate progeny (sex 4-5-6) and 204 female progeny (sex 7-8-9).

It was observed that there were only 27 no-tasseling varieties. In a general way only few variations have been observed from one year to the other related to natural tasseling and sex rating. This is possibly due to regularity on the climate.

Tasseling periods:

up to 4 May	early
from 5 to 13 May	medium early
from 14 to 22 May	medium
from 23 to 31 May	medium late
from 1 to 9 June	late
from 10 to 19 June	late reluctant
from 20 onwards	reluctant

TASSELING CONTROL

Research referring to tasseling control is carried out to cause the floral initiation of varieties which even after several years of cultivation have not yet tasselled (Allard[1]). On the other hand they provide the possibility of altering the period of tasseling to allow crossings of progeny which normally tassel at different periods (Coleman[2]).

In order to cause the differentiation, application to the soil of several chemical products such as calcium silicate and sodium borate has been tried out. Ethrel and Gibberelinehave been used for leaf spraying. Based on the principle that the reluctant varieties need a longer dark period in order to produce the floral stimulus, an

experiment in natural conditions was carried out (James[5]).

Even after a several year lasting period of adaptation to the cultivating conditions in Camamu, certain varieties had not yet tasselled naturally up to then (Georges[3]). Therefore, one collection was planted along the border of a wood so that the shade of the trees would simulate the sunset. In 1978, by this mean, it was possible to obtain the tasseling of the following varieties: B51415, B55227,B55362, B59163, B60191, BJ5924, D117, DB136/56. Varieties with a long vegetative cycle, as the Hawaiian ones, are only able to receive floral induction after more than one year of vegetative cycle or sometimes in ratoon.

In order to delay the tasseling of the early tasseling progeny, the ratoons are cut back between August and October. A second cutting back is made in December. This method requires the plantation of the sugarcane in separate collections, according to each period of tasseling.

The method of leaf cutting (Julien[6]) was used as well. It was carried out at the beginning of January, before the floral induction of the early tasseling varieties (Moore[11]). In the first operation, the leaves were cut just a little above the apical stem. Twenty days later, before the leaf spindle attains its complete development, a second leaf cutting takes place just below the last visible dewlap. These two cuttings delay the period of the floral initiation until the end of February. Some varieties showed a development of the lateral stems weakening the tasseling.

After some experiments on the interruption of the dark period (Latouette[7]) it was observed that the tasseling of the earliest variety of the collection, P57-150-4, could be avoided altogether. For this purpose, a tungsten lamp of 100 W was used for two hours, from 8 p.m. to 10 p.m., from January till March. The best progeny were planted in the shape of a star with eight beams. On each beam are planted 10 holes of one progeny. The lamp was placed at a height of five meters in the center of the "star".

By means of this process it is possible to obtain tassels at various periods. The holes situated at a greater distance from the source of illumination tassel earlier, while the holes placed directly under the lamp produce tassels later. This method has produced excellent results under the existing conditions. In 1978, by means of these different methods of tasseling control, it was possible to coincide at the same time the tasseling of 607 progeny from a total of 879 progeny used in the crossing campaign.

FUZZ TREATMENT

The fuzz is separated from the spikes of the tassels and put into a dryer at a temperature between 35°C and 40°C in order to remove humidity excess. Afterwards all the fuzz is processed by the method da Silva[12,13] and stored with silica gel in hermetically closed drums. Low humidity rate is very important to the preservation of the germination rate of seeds. During the last years the drying has been improved a great deal. A freezer is no longer used. Seeds preserved at ambient temperature in closed containers with silica gel have germinated after five years.

COPERSUCAR SELECTION SCHEME

Crossing in Camamu
De-fuzzing and storage
Sowing in Sao Paulo Experiment Stations

Space planting — Continuous planting

Ist selection — ratoon of seedlings

2nd selction — Resistance to diseases
3rd selection — Multiplication
4th selection — Trials in sugarmill fields
5th selection

Plant cane — Ratoon

Clone trials in the State of Sao Paulo
6th selection — Plant cane and ratoon
Results of official smut testing
Release of SP varieties

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EL PROGRAMA DE FITOMEJORAMIENTO DE COPERSUCAR.

Autor: J. Y. J. Mioque, San Pablo, Miocque.

RESUMEN

El programa de mejoramiento genetico de Copersucar, fué iniciado en 1968 con la instalacion de la Estacion Experimental de Camamú en el Estado de Bahiá, para la obtención de semilla de caña. La germinación de la semilla y la seleccion clonal se realizan en estaciones experimentales en el Estado de San Pablo, bajo las condiciones ecológicas en que deberán cultivarse las futuras variedades SP.

Las condiciones climáticas de Camamú son especialmente favorables para la floración de la caña de azúcar y para la producción de semilla botánica. La cantidad de semilla producida permite satisfacer las necesidades de varios institutos de investigacion. Por medio del control de la floración, se hace posible coincidir el momento de floración de un gran numero de progenies al mismo tiempo. La viabilidad de la semilla se incrementa por medio de variados tratamientos. Las primeras 6 variedades SP, resistentes al carbón y al mosaico, fueron liberadas al cultivo en 1978.

GENETIC DIVERGENCE IN SUGARCANE

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ABSTRACT

A group of 48 varieties of sugarcane collected from different geographical sources was studied to understand the nature of genetic divergence using Mahalanobis's statistical distance. Varieties having genetic similarity were grouped in 8 clusters. Grouping of varieties in different clusters was not related to their geographical origin. The maximum inter-cluster distance was observed between VII and VIII followed by II and VIII. Cluster VII was much diversed than others. Varieties belonging to different clusters having maximum genetic distance were promising and recommended for hybridization program to improve yield and quality.

INTRODUCTION

The knowledge of nature and degree of divergence in inter-and intra-allelic level is useful for understanding the course of evolution in new varieties. It is also useful in selecting desirable parents for breeding program, since it is known that exploitation of heterosis and success in obtaining desirable recombinants is dependent on degree of divergence of the parents. The use of diverse germplasm as significant factor contributing to high yield has been stressed by many workers (Murty and Anand[3] Singh, and Bains[4], Singh *et al*[5], Singh and Singh[6]). This technique has very meagerly been documented in this crop. The present study was undertaken for comparing 48 strains of sugarcane together by clustering them into groups using D^2 statistics.

MATERIALS AND METHODS

Forty-eight strains of different origin were selected from a large germplasm maintained at U.P., Council of Sugarcane Research, Shah jahanpur. These were grown in randomized block design with three replications.

Each variety had three rows, 12 m long and 90 cm apart in each replication. The data were recorded on 10 competitive clumps for number of millable canes/clump, stalk height (m), stalk girth (cm), number of internodes/stalk, number of green leaves/plant, brix, weight of cane (kg) and top weight (kg).

The plot means were used for statistical analysis. Differences among varieties were tested by means of variance for all traits. The grouping of varieties was done according to procedure given by Rao *et al.*

RESULTS AND DISCUSSION

The forty-eight varieties were grouped into 8 clusters. The approximate configuration of the eight different clusters is given in Fig. 1. Varieties within cluster had smaller D^2 value among themselves than those belonging to different clusters (Table 2). Two largest clusters were II and III containing 14 and 13 varieties respectively followed by clusters IV, V and VI. The clusters I and VIII each contained two varieties each from Coimbatore. Cluster VII contained two varieties only, one from Shah jahanpur and second from Coimbatore.

Intra-cluster distance ranged from 21.05 to 82.70. The highest was observed for cluster VII, followed by clusters II and VIII. Inter-cluster distance ranged from 24.89 to 112.34. The clusters VII and VIII had maximum diversion followed by clusters II and VIII. Clusters II, III, IV and V were quite close to each other.

Cluster mean (Table 3) indicated that cluster II had highest brix and next to highest weight of cane, no. of internodes/stalk and height which separated this cluster from remaining clusters. The cluster VII containing two varieties each from Coimbatore had highest weight of cane (1.109) and height (2.95). It had also next to highest brix (17.50). It indicated that diversity in this cluster was due to the height of cane and weight of cane. Clusters III and IV had medium mean values for each character.

The pattern of 8 clusters observed indicated that the clustering pattern of different varieties did not necessarily follow their geographical distribution and was fairly random. Varieties from Coimbatore dispersed in all 8 clusters while varieties of Shah jahanpur (Co.S.) and Bihar (B.O.) distributed in V and VI clusters, respectively. The pattern of varieties occurring in clusters cutting across geographical boundaries clearly demonstrates that geographical diversity need not be related to genetic diversity. Arunachalam and Jawahar Ram[1] in Sorghum *(Sorghum bicolor* (L) Moench), Murty and Anand[3], and Singh and Bains[4] in cotton *(Gossypium hirsutum* L.) also found that there was no relationship between geographical diversity and genetic diversity. It may, therefore, be concluded that varieties with the same geographical origin might have different genetic background and wide divergence in features. Inspite of geographical separation other forces are also responsible for divergence. Genetic drift and selection in different environment could cause greater diversity than geographical distance (Murty and Anand[3], Singh and Bains[4], Singh and Singh[6]). Endang[2] suggested that varieties belonging to different clusters having maximum distances are to be selected for hybridization so that the highest possible variability can be generated in the yield and quality components.

SUMMARY

A group of 48 varieties of sugarcane collected from different geographical sources was studied to understand the nature of genetic divergence using Mahalanobis's statistical distance. Varieties having genetic similarity were grouped in 8 clusters. Grouping or varieties in different clusters was not related to their geographical origin. The maximum inter-cluster distance was observed between VII and VIII, followed by II and VIII. Cluster VII was much diversed than others. Varieties

TABLE 1. Analysis of Variance for different characteristic in sugarcane

Mean sum of squares

Source of Variation	D.F.	No. of millable canes/ clump	Stalk height (M)	Stalk girth (cm)	No. of inter-nodes/	No. of green/ leaves/	Brix	Stalk weight (kg)	Top weight (kg)
Varieties	47	3.330**	2365.283**	0.200**	16.023**	1.535*	14.417**	17.410	3.312
Error	94	1.284	253.162	0.029	1.619	1.032	2.098	16.275	3.301

Significant at 5% level of probability.
Significant at 1% level of probability.

TABLE 2. Intra and Inter-cluster average divergence (D^2) values of the 8 clusters of sugarcane.

	I	II	III	IV	V	VI	VII	VIII
I	22.37 (4.73)	31.49 (5.61)	40.99 (6.40)	37.99 (6.16)	51.65 (7.18)	55.35 (7.44)	75.40 (8.68)	92.38 (9.61)
II		22.96 (4.79)	25.03 (5.00)	29.61 (5.42)	36.82 (6.06)	40.63 (6.37)	51.16 (7.15)	102.75 (10.13)
III			21.05 (4.58)	26.69 (5.46)	25.14 (5.01)	34.99 (5.78)	48.84 (6.99)	92.81 (9.63)
IV				25.70 (5.07)	27.29 (5.020)	37.17 (6.09)	60.41 (7.70)	76.13 (8.72)
V					24,89 (4.98)	39.86 (6.31)	62.67 (7.91)	80.89 (8.99)
VI						51.23 (7.15)	62.49 (7.90)	95.04 (9.70)
VII							82.70 (9.09)	112.34 (10.59)
VIII								69.50 (8.33)

Data in parenthesis show the corresponding square roots, e.i. "D" values.

belonging to different clusters having maximum genetic distance were promising and recommended for hybridization program to improve yield and quality.

TABLE 3. Character means of different clusters in sugarcane

Cluster	No. of millable canes/ clump	Stalk height in M	Stalk girth in cm	No. of inter-nodes/ stalk	No. of green leaves/ plant	Brix	Stalk weight in kg	Top weight in kg
I	6	2.29	2.14	24	11	17.80	0.902	0.188
II	5	2.50	2.10	23	9	18.77	1.004	0.130
III	6	2.47	2.05	22	8	16.70	0.870	0.120
IV	6	2.46	1.82	22	9	16.89	0.725	0.115
V	5	2.37	1.90	20	7	15.30	0.810	0.082
VI	6	2.50	1.70	24	7	14.90	0.805	0.135
VII	5	2.95	2.17	24	8	17.50	1.109	0.106
VIII	8	1.69	1.68	21	9	15.66	0.493	0.128

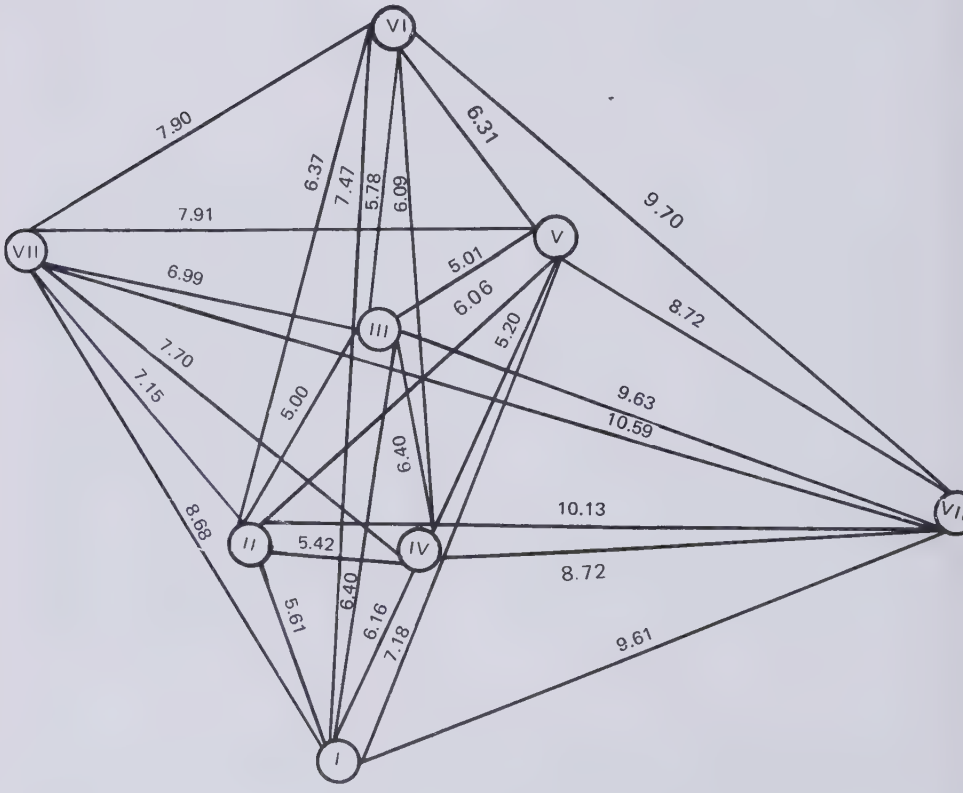

FIGURE 1. Approximate configuration of the eight different clusters of sugarcane varieties. Circle indicate clusters and lines indicate inter-clusters distances.

TABLE 4. Distribution of the 48 sugarcane varieties in different clusters.

Cluster	Varieties
I	Co 853 Co 975
II	Co 62399 CoS 611 Co 1347 Co 846 Co 1148 Co 1158 Co 453 CoS 109 Co 313 Co 421 Co 6611 Co 1007 Co 1336 CoS 792
III	CoS 659 Co 6812 Co 1149 Co 6911 Co 6425 Co 285 CoS 673 Co 1157 B.O. 34 Co 213 CoS 510 CoS 658 Co 331
IV	Co 370 B.O. 32 Co 859 B.O. 47 CoS 633
V	Co 527 Co 1111 Co 1125 B.O. 70 B.O. 54
VI	B.O. 17 CoS 771 CoS 770 Co 62035 CoS 541
VII	CoS 758 Co 6914
VIII	Co 395 Co 205

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DIVERGENCIA GENETICA EN CAÑA DE AZUCAR

H. N. Singh y S. B. Singh

RESUMEN

Un grupo de 48 variedades de caña de azucar coleccionadas en diferentes areas geograficas, sirvio de base para investigar la naturaleza

de la divergencia genetica entre las mismas, haciendo uso para ello de las distancias estadisticas de Mahalanobis. Las variedades con similitud genetica fueron agrupadas en 8 conglomerados. La agrupacion de variedades no estuvo asociada al origen geografico de las mismas. Las maximas distancias intergrupales fueron observadas entre VII y VIII y despues entre II y VIII. El grupo VII fue mas diverso que otros.

Las variedades pertenecientes a diferentes conglomerados que mostraban distancias genetivas maximas fueron promisorias y recomendadas para los programas de hibridacion con el objeto de incrementar rendimiento y calidad.

FURTHER STUDIES ON SUGARCANE POLLEN

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ABSTRACT

To gain more knowledge on the behavior and structure of sugarcane pollen, viability tests were conducted and the scanning electron microscope photography were used for pollen collected at its shedding period.

The results of the study confirmed findings of previous experiments (Krishnamurthi[3]) which showed that sugarcane pollen is viable only for one hour, it is highly sensitive to humidity and light, and that it absorbs nutrients from stylar tissue to penetrate and grow enough style.

INTRODUCTION

Sugarcane pollen has been the subject of very few studies. In fact, little has been known on the entire developmental process although sugarcane breeding has been going on for almost a century. The female gametophyte was studied by Narayanswami[5] in a limited manner. Pollen development has to some extent been observed but embryo development was totally neglected.

The viability and preservation of sugarcane pollen was studied by Krishnamurthi[3], King[2] and Sartoris[7]. The ability of sugarcane pollen tube to penetrate the stylar tissue was also reported by Krishnamurthi[4], Nair and Narasimhan[6]. Structures on the preservation of pollen was conducted to enable crossing between varieties which flower at different times. While it is possible to preserve pollen for 24-48 hours. Beyond this period pollen preservation appears to be difficult. Any temperature below 1°C appears to be fatal (Krishnamurthi[4]). This study aims to gain further insight into the mechanism to the pollen behavior and structure.

MATERIALS AND METHODS

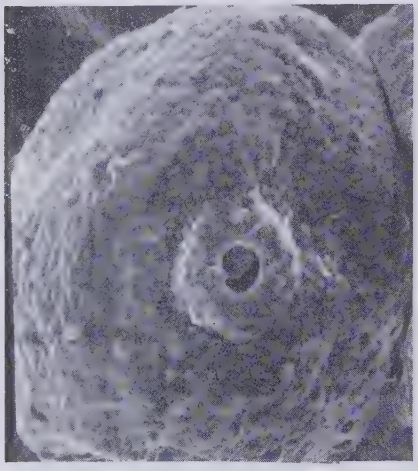
Sugarcane pollen in Fiji is shed normally at 7:15 a.m. It was collected at this time using large sheets of paper. Then, the collected pollen were sieved and stored. Pollen for viability tests were cultured in a medium made for 300 g calcium nitrate, 100 mg boric acid, 100 mg magnesium sulphate and 300 g sucrose in a liter of water.

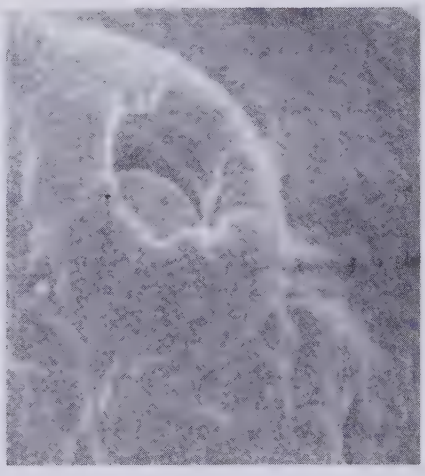
Pollen was stored dry for further studies. The sugarcane pollen normally

shrinks when it is dry therefore, it should be subject to high humidity before treatment for photography, so that it is inflated and round. This was done simply by mildly blowing by mouth. The pollen for scanning electron microscope photography was impregnated with gold vapor and photographed by Hitachi equipment at the University of California, Irvine by Dr. M. Strause.

RESULTS

The Plates 1 and 2 show a number of features which were not known about sugarcane pollen. The surface was thin and relatively smooth compared to dicot pollen which has innumerable projections. It was also made of circular convex discs with a large number of dots which were probably perforations. The configuration and architecture were simple. The lip area was raised in the form of a mound with the opening for the tube emergence. This opening was oval in shape and placed in the center.





The lip appeared to be curled giving the appearance of a double layer. Through the opening or the orifice which is oval in shape the pollen tube primordia giving the appearance of a tongue could be seen. The pollen tube primordia was also dotted. The tube appeared to be attached to one side with a clear opening on the other. It was likely that this open space would allow aeration and entry of fluids — possibly, nectar.

DISCUSSION

The sugarcane pollen has the following characteristics (Krishnamurthi[3]):

a. It is viable only for one hour.
b. It is highly sensitive to humidity. Low humidity causes it to die quickly.
c. It is sensitive to light. It grows well under lighted conditions.
d. It absorbs nutrients from stylar tissue to penetrate and grow through the style (Brewbaker[1]).

The above mentioned conclusions were based on observations made on the SEM pictures taken.

The wall of sugarcane pollen was much thinner than the walls of many other pollens. The gold impregnated dots were probably fine perforations which allow the passage of air and nutrients. This possibly could help the pollen to attach to the stylar tissue. The perforations and the thin wall make the pollen dependent on environment for viability and ability to germinate.

Due to such perforations on the wall, the water loss was great and quick. Under low humidity, the pollen dessicated very quickly, in fact, within a matter of minutes, hence very low fertility rates occurred where low humidity existed. When exposed to free water, the pollen imbibed water and burst discharging its contents. However, they survived well under 90-98% R.H.

The pollen tube primordia grew quickly. Within 10 minutes, the tubes were already visible with the movement of the cytoplasm and starch grains within the tube. The fact that the tube was also perforated is a good indicator that it absorbed nutrients as it grew, and that it grew relatively fast under optimum conditions. The opening on the side of the tube primordia was probably a point of entry for the nectar from style to trigger the germination of the pollen.

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NUEVOS ESTUDIOS SOBRE EL POLEN DE LA CAÑA DE AZUCAR

M. Krishnamurthi

RESUMEN

El polen de la caña de azucar es viable solamente durante una hora en condiciones naturales. Algunos metodos desarrollados hasta ahora permiten su preservacion durante dos dias. A efectos de estudiar la ultra-estructura del polen, éste fué fotografiado con el microscopio electronico de exploracion ("Scanning"). Este estudio revela la presencia de paredes finas repletas de finas perforaciones.

SUGARCANE PROTOPLAST

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ABSTRACT

Protoplast culture offers new possibilities to produce hybrid material asexually. It will also enable plant breeders and geneticists to modify plant characteristics through gene modification. However, culture of the protoplast is a difficult process. The reasons behind the difficulties encountered in protoplast culture have been studied with the help of scanning electron microscopy.

INTRODUCTION

Protoplasts have attracted much attention recently, resulting in an increasing number of researches on various aspects of the protoplast. Parasexual hybridizations seems to occupy a top position on the list. The feasibility of genetic transformations through protoplast culture the protoplast's reactions to virus, and the capacity of the cell to synthesize various chemicals are some of the concerns of the researches.

While isolation of protoplasts is reasonably easy, the culture and differentiation of plants appear to be rather difficult. So far, most reports acknowledge success with Nicotiana (Melchers[4] and Gamborg[2]). There are very few reports of success with other plants, Baraer[1] reported success with the callus development in the fusion products of *Zea x Sorghum.* This callus however, would not differentiate into plants.

Sugarcane protoplasts were first isolated by Maretzki and Nichell[5] using helicase and other enzymes. Krishnamurthi[3] described the isolation and fusion of sugarcane protoplasts using a technique developed by Kao *et al.* Since then, we have gained more experience on protoplast culture.

MATERIALS AND METHODS

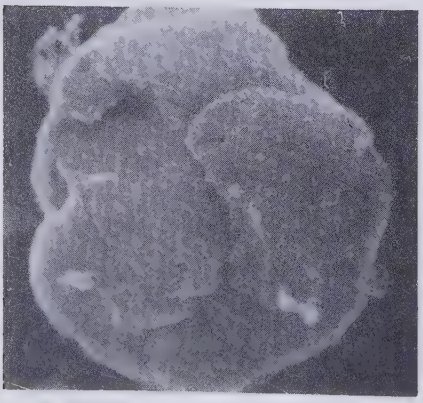
Sugarcane *(Saccharum officinarum)* sets were grown in three liter planters and supplied with abundant nutrients and adequate water for lush growth. The required tops were cut wrapped in polythene paper and left in the dark for at least twelve hours prior to the digestion of the spindle for the extraction of protoplasts.

The spindle was dissected and the soft 5 cm core was taken with the dome (Krishnamurthi[3]). The thickness was based on the softness of the tissue. This was placed in 200ml/liter commercial hypochlorite solution and transferred to a bio-clean station. All further operations were carried out under asceptic conditions. The spindle tissue was sterilized for 20 minutes and then washed free of hypochlorite solution with sterile distilled water. Both ends were cut and layers of primary leaf tissues peeled off until the last whorl was left. This was chopped finely and placed in medium 'A' containing 5mg/ml cellulase, 2 mg/ml macerozyme (both Onosuka R10) 6m mol/lit calcium chloride; 0.7m mol/lit sodium dihydrogen phosphate; 0.7m/lit, glucose, 0.1 mg/ml sodium diethyl-dithia-carbamate, 10mg/lit dextran sulphate, 20mg/lit calcium sucrose phosphate. The medium 'A' with enzymes was filtered and sterilized with millipore membrane filter. The tissues were allowed to incubate for 180 to 240 minutes at which time, most of the walls were digested. The tissues were smashed with glass rod, filtered through fine nylon mesh screen and washed freely with medium "B" (Appendix 1). This was done by placing the protoplasts in embryo blocks and adding medium 'B' and removing with a pipette. The protoplasts were cultured in well aerated containers for good results

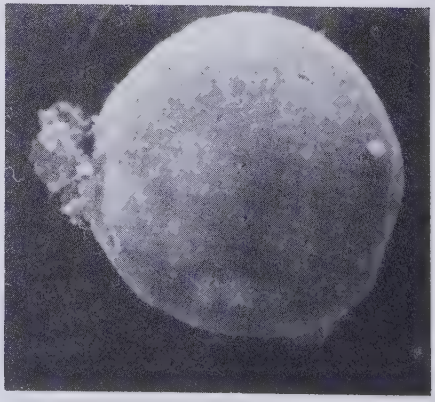
The normal pictures were taken under ordinary light microscope in the laboratories. The scanning electron microscope pictures were made by Dr. M. Strause and Prof. Arditti of the Dept. of Cell Biology University of California, Irvine, USA. The protoplasts were fixed in 2% gluteraldehyde, and shipped to California where SEM pictures were taken.

RESULTS

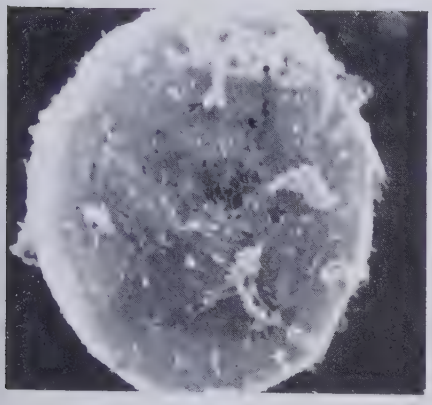
The previously used enzyme mixture in medium 'A' (Krishnamurthi[3]) was found to be so strong causing over-digestion which killed the protoplasts. With the current mixture, the digestion time was prolonged. The polyphenols which normally act as suppressors of cell-division were neutralized by the addition of sodium diethyl-dithiacarbamate. Aeration of the protoplasts was very critical as it deter-



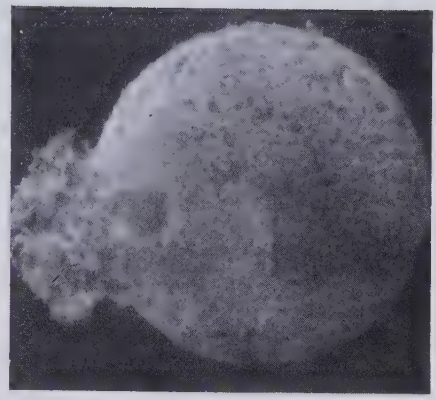
1. Cell poll digested lining membrane intact.



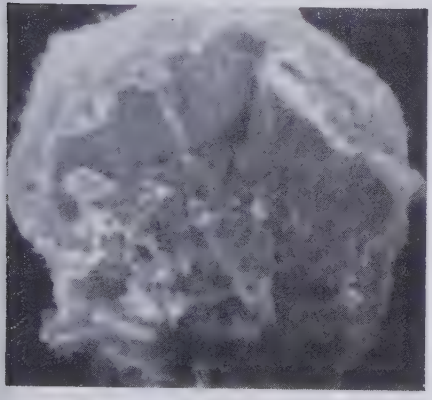
2. Commencement of damage to protoplast second membrane.

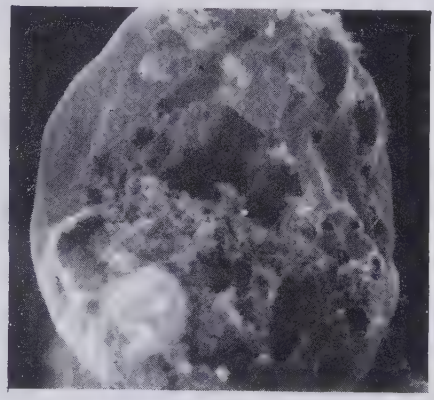


3. Pitting in the center.



4. Damaged second layer membrane.





5-6. Extensive damage to second and third layers. Various internal organelles can be seen.

mines the viability of protoplasts.

The protoplasts showed very little as to the fate of the cell membranes when examined under normal light microscope. However, the scanning electron microscope revealed the various stages of digestion as can be seen in the following plates.

DISCUSSION

Protoplasts have attracted much attention with regards to creating parasexual hybrids and modifying genes with the introduction of DNA modifiers. However, success so far has been limited due to the ability of the protoplasts to regenerate divide, form callus and differentiate into plants. Also it has been clearly established what happens to the chromosomes from the two cells. The compatibility and rejection phenomena is yet to be demonstrated. Some of the observations made on the phenomenon shown in the scanning electron microscope pictures shall be discussed.

The plant cell wall which includes the cellulose section, the membrane which is possibly made up of proteins and a much thinner second membrane which covers the content of the protoplasts are all very complex living matter which in an actively dividing cell continually interact with the cytoplasmic matter. When the actively dividing cells were subjected to enzyme action the cell wall started to dissolve.

The callus cells, on the other hand which were actively dividing were also generally resistant to enzyme action. There were however, cells in the callus mass, which were round in shape and subject to enzyme action. These cells would yield protoplasts.

From step by step study of the wall digestion, there appeared to be three distinct layers of wall material. The outermost layer was made up of cellulose which was digested by cellulase. The second layer was probably made of proteins and other substances which were digested only by the combined action of pectinase, cellulase and hemicellulase. Immediately below this layer was the transparent membrane-like substance which held the protoplast intact.

From the SEM photographs, it can be seen that once the second layer was damaged, the inside of the cells were exposed to the nutrient medium. The medium, when in direct contact, naturally proved physiologically incompatible with the content of the cytoplasm and therefore the cells succumbed to death quickly. The damage to the second layer was caused either by prolonged incubation period of not thoroughly washed enzymes. In the second case, the presence of minute quantities of enzymes, continued reactions and caused pitting on the second layer, thus creating weak points on the wall. Usually at such points, the cytoplasmic matter oozes out and subsequently bursts ending up in total kill. Therefore, to obtain viable protoplasts, a number of conditions have to be critically controlled. Some of them are – time of digestion, washing of enzymes, aeration, culture conditions, light intensity and pre-treatment of tissues prior to extraction of protoplasts.

Appendix 1

Culture Medium 'B'

The culture medium is made as follows:

Stock solutions were made from which the indicated amount is taken and made up to a liter with distilled water. The liquid medium was either filter sterilized or autoclaved.

Chemical	Stock Solution grams/lit A–F	Soluble in	Final composition ml/liter

A.	Ammonium nitrate	82.00	water	20
	Ammonium sulphate	27.00		5
	Potassium nitrate	95.00		20
	Calcium chloride	88.00		20
	Calcium nitrate	60.00		5
	Calcium dihydrogen phosphate	20.00		5
B.	Boric acid	1.25	water	
	Potassium dihydrogen sulphate	34.0		
	Cobalt chloride	0.005		5
	Sodium molybdate	0.050		
	Sodium dihydrogen phosphate	15.00		
	Potassium iodide	0.002		
C.	Magnesium sulphate	74.0	water	
	Zin. sulphate	1.72		
	Cupric sulphate	0.005		
	Manganese sulphate	2.50		
	Chemical	Stock solution grams/lit A–F	Soluble in	Final composition m/liter
D.	Ferrous sulphate	6.00	Hot water	5
	Sodium EDTA (or sequistic acid)	7.4		
E.	Coconut water		Add direct	20 ml
F.	Cassein hydrolysate		Add direct	0.25 g
	Sucrose			25 g
	Xylose			0.50 g
	Ribose			0.50 g
	Dextrose			1.00
	Fructose			1.00
	Glucose			140.0

Calcium sucrose phosphate 10 mg — 0.101 g

	Chemical	Stock solution wt/lit A-F wt/100ml G-P	Soluble in	Final composition ml/liter
G.	2, 4-D	200 mg	99% alcohol	1
	IBA	200 mg	"	1
	NAA	200 mg	"	1
H.	Kinetin	500 mg	NaOH	1
I.	Thiamin	5.5 g	Water	
J.	Niacin	10 mg	Water	
	Pyridoxion	100 mg	Water	1 ml
	Glycine	100 mg		
	Riboflavine	200 mg		
K.	Inositol	10,000 mg (10g)	Water	1 ml
	Ascorbic Acid	20,000 mg (20g)	Water	1 ml
	Biotin	1 mg	Water	
	Caffein	1 mg		1 ml
	Choline chloride	100 mg		

	Chemical	Stock solution wt/100ml G-P	Soluble in	Fine composition ml/lit
	Folic acid	40 mg		
	p-aminobenzoic acid	2 mg		
	Calcium penthothenate	100 mg		
L.	Vitamins A _____	1 mg	Water	
	B_{12} _____	1 mg		1 ml
	D_3 _____	1 mg		

M.	Arginine	1 g	Water	
	Histidine	10 g		
	Lycine . . . water soluble	5 g		
	Leucine	1 g		1 ml
	Methionine	1 g		
	Phenylalanine	1 g		
	Threonine	1 g		
N.	Tryptophane . . . soluble	1 g	NaOH	1 ml
O.	Valine	1 g		
	Aspartic Acid . . . HCl	1 g		
	Asparagine	1 g	HCl	1 ml
	Serine	1 g		
	Tyrosine	1 g		
	Cystine	1 g		
P.	Glutamic acid hot	1	Hot water	
	Proline . . . H_2O	1		
	Alanine	1		1 ml
	Adenine	1		
	pH	5.8		

Adjust pH to 5.8 by addition of NaOH or HCl as the case may be.

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EL PROTOPLASTO DE LA CAÑA DE AZUCAR

M. Krishnamurthi

RESUMEN

El cultivo de protoplastos ofrece nuevas posibilidades para la obtencion de material hibrido sin sexo. Esto permite tambien a los fitomejoradores y geneticos a lograr modificaciones en la planta a traves de modificaciones en los genes. El cultivo de protoplastos es un proceso dificil sin embargo. Las causas han sido estudiadas por microscopio electronico de exploracion ("Scanning").

RELATIONSHIP OF POLLEN AND PISTIL CHARACTERISTICS TO SETTING OF TRUE SEEDS IN SUGARCANE CROSSES

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ABSTRACT

A study was conducted at Houma, Louisiana, to investigate the association between setting of true seed and the characteristics of floral organs in sugarcane crosses.

A positive correlation was found between breeding generations, size and variability of pollen grains. The more highly mobilized the generation, the larger and the more variable were the pollen grains.

Seed sets increased exponentially with pollen maturity of male parents and was closely associated with anther dehiscense and shedding of pollen.

Tubes of pollen grains germinated in artificial media, when compared with pistil parts, appeared to be too short for fertilization.

Better seed sets can be expected in interspecific crosses when mature pollen grains of male parents exceed 60% and the stigmas of female parents are fully expanded outside the glumes.

Seed sets in intergeneric crosses were apparently governed by sexual incompatibility of the parents rather than by characteristics of the floral organs. Certain possibilities of attaining intergeneric crosses more easily are discussed.

INTRODUCTION

Wide variation in the setting of true seeds among different sugarcane crosses is frequently noted. Certain environmental and genetic factors, acting independently or in combination with each other, are known to affect the production of true seed (Akhtar *et al*[1], Brett[2,3], Dunckelman[6] Natarajan *et al*[18], Paliastseas and Chilton[19], and Stevenson[21]). Low temperature adversely affects uniformity of pollen grains, opening of the anthers and fertility of the ovule, Consequently, crossing sugarcane under controlled temperature is specialized facilities has resulted in excellent production of true seeds, especially in sub-tropical countries (Brett[2] and Dunckelman[6,10]). A short daylength influences anther dehiscence, pollen maturity and ovule maturity (James[15] and Natarajan *et al*[18]). Male-sterile parents are particularly sensitive to male fertility if the treatment is continuous after floral initiation is established (James[15]). In a number of crosses incompatibility exists because of genetic, cytoplasmic and organic conditions in pistil parents, and these appear to exert an influence on the viability of hybrid seeds from some varieties (Stevenson[21]).

The mechanics of true seed setting in sugarcane are complicated. The present study was conducted to demonstrate major characteristics of floral organs and their association with seed setting in basic sugarcane crosses made at Houma, Louisiana during the 1976 breeding season.

MATERIAL AND METHODS

Flowering parts for microscopic examination were taken from two tassels of all varieties just before setting in crosses. To determine percentages of mature pollen grains, five anthers from each of two tassels were crushed on a glass slide and stained with a 5% potassium iodine solution. Ten replications of each type of pollen grain were counted under a 100x field. Pollen grains were considered "mature" only if they were stained completely black, and percentages of completely mature grains were calculated accordingly. The diameter of fifteen mature grains of each sample were measured in order to obtain average size of grains and their coefficient of variation. For observations on growth of pollen tubes, grains fresh from dehiscent anthers were cultured on hanging drops of Moor's[17] media on petri plate covers at 21°C, and were fixed in 75% alcohol after 6 hours. The length of pollen tubes was measured from micro-photographs taken under 40x microscopy. In the case of pistil parents, stereomicroscopic observations were made under a 20x field of measure ovule size, stigma length, and expanse of stigma which stretched outside glumes.

Number of viable seeds per cross was estimated by counting seedlings per gram of fuzz taken at random from each cross. Greater accuracy in determining viability of true seeds was later determined by counting the total number of seedlings produced from "en masse" plantings of carefully weighed amounts of fuzz from each cross.

RESULTS AND DISCUSSION

Characteristics of pollen grains. Under microscopic examination, it was noted that mature pollen grains were stained completely black, partially mature ones incompletely black. The immature grains showed no effect from the stain. The frequency of mature grains, their average size and coefficient of variation (C.V.) according to each breeding generation are summarized in Table 1. The maturity of grains within each generation was highly variable with about one-third of 77 varieties showing more than 60% maturity. In general, all of the *Saccharum spontaneum* L. clones, with the exception of SES 189, produced a high percentage of mature pollen grains. Apparently, SES 189 also transmitted low staminal fertility to its progenies. Sixteen of 17 F_1 generation hybrids from SES 189 showed low pollen maturity as shown in Table 2-4. Many of the important commercial breeding varieties in Louisiana also fell into the category of a low percentage of mature pollen grains, indicating a high degree of male sterility (Table 2-6).

TABLE 1. The frequency of the pollen grain maturity and average size and c.v. of the grain in each generation.

Generation	Maturity of grain (%) 0 ∽ 20	20 ∽ 40	40 ∽ 60	60 ∽ 80	80 ∽	Total	Av. size of grain (μ)	Av. c. v. of grain (%)
Noble	0	1	0	0	0	1	53.9	13.3
Ripidium sp.	1	0	0	1	0	2	37.0	6.5
S. Spontaneum	1	0	0	9	5	15	38.0	7.5
F_1	17	3	4	3	1	28	44.4	8.4
BC_1	4	2	0	4	1	11	45.1	9.5
Commercial	8	5	5	1	1	20	48.0	9.2
Total	31	11	9	18	8	77		
Ratio (%)	40.2	14.3	11.7	23.2	10.4	100		

TABLE 2-1: *Ripidium* Species

Species	Variety	Date	Grains obs'd	Mat'd grain (%)	Av. size grain (μ)	c.v. grain (%)
R. bengalense	US 59-10-2	Nov. 1	780	76.5	37.0	6.50
R. kanashiroi	Unknown	Nov. 1	236	0.7	—	—

TABLE 2-2. *Saccharum spontaneum*

Variety	Date	Grain obs'd	Mat'd grain (%)	Av. size grain (μ)	c.v. grain (%)
SES 96	Oct. 9	473	94.5	37.6	4.45
SES 6	Nov. 19	782	94.4	35.9	7.47
Mandalay	Oct. 9	426	89.9	40.3	5.96
SH 246	Oct. 9	384	84.9	37.5	6.95
SES 205A	Oct. 9	334	81.7	39.4	10.00
Djotiroto	Nov. 12	485	79.8	36.1	9.91
SES 343	Nov. 1	432	78.2	40.7	7.76
SES 208A	Oct. 9	625	77.6	37.6	6.25
Soembawa	Nov. 23	310	75.4	36.5	5.88
SES 231	Oct. 9	452	74.3	41.3	8.75
SES 50	Nov. 17	498	71.3	37.2	8.36
US 56-15-8	Dec. 6	620	71.1	40.6	14.81
SES 577	Nov. 19	396	66.9	35.4	6.07
US 56-15-2	Nov. 17	606	64.2	35.9	10.06
SES 189	Oct. 9	271	10.0	—	—

TABLE 2-3. *Saccharum officinarum* (Noble cane)

Variety	Date	Grains obs'd	Mat'd grain (%)	Av. size grain (μ)	c.v. grain (%)
Fiji 40	Oct. 26	399	31.6	53.9	13.29

TABLE 2-4. F_1 generation

Variety	Line [1]	Date	Grains obs'd	Mat'd grain (%)	Av. size grain (μ)	c.v. grain (%)
US 74-103	SES 205A	Nov. 29	795	83.0	44.1	10.99
US 74-103	SES 205A	Nov. 1	631	68.5	44.0	9.27
US 72-189	SES 147B	Nov. 8	658	66.4	45.1	8.14
US 72-247	US 59-1-1	Nov. 12	519	62.8	46.4	7.22
US 74-103	SES 205A	Nov. 23	461	57.9	44.3	8.54
US 72-185	SES 147B	Nov. 17	773	48.4	47.3	9.93
US 72-201	SES 189	Nov. 23	373	44.0	44.1	7.67
US 72-187	SES 147B	Nov. 8	661	41.1	42.6	5.52
US 72-223	SES 189	Nov. 3	447	24.8	43.9	8.85
US 72-192	SES 189	Nov. 3	560	24.0	43.8	6.81
US 72-199	SES 189	Nov. 3	754	20.7	43.1	9.90
US 72-214	SES 189	Nov. 4	656	19.7	–	–
US 72-221	SES 189	Nov. 2	368	18.5	–	–
US 72-167	SES 189	Oct. 26	460	11.3	–	–
US 72-211	SES 189	Oct. 27	383	9.9	–	–
US 72-166	SES 189	Nov. 3	229	6.6	–	–
US 72-168	SES 189	Nov. 4	500	4.6	–	–
US 73- 55	SES 124	Nov. 22	581	4.6	–	–
US 72-178	SES 189	Nov. 17	675	2.1	–	–
US 72-192	SES 189	Nov. 2	394	0.3	–	–
US 72-213	SES 189	Oct. 26	466	0.2	–	–
US 72-212	SES 189	Oct. 26	479	0.2	–	–
US 72-213	SES 189	Nov. 12	327	0	–	–
US 72-162	SES 189	Nov. 8	147	0	–	–
US 72-212	SES 189	Nov. 15	250	0	–	–
US 72-165	SES 189	Nov. 3	38	0	–	–
US 72-231 [2]	SES 189	Nov. 1	–	–	–	–
US 72-231 [2]	SES 189	Dec. 6	–	–	–	–

[1] *All varieties of the line are* S. spontaneum.

[2] *Complete anther deficiency.*

TABLE 2-5. BC_1 generation

Variety	Line [1]	Date	Grains obs'd	Mat'd grain (%)	Av. size grain (μ)	c.v. grain (%)
US 73-39	US 56 14-4	Nov. 30	826	92.1	49.2	7.08
US 73-28	Gehra Bon	Nov. 19	692	74.3	45.0	10.43
US 73-32	Gehra Bon	Dec. 3	707	71.1	40.8	4.45
Benda Sel.[2]	Unknown	Nov. 26	865	65.4	45.5	10.76
US 73-68	SES 147A	Nov. 18	513	61.8	46.7	19.86
US 72-157	SES 92	Nov. 8	958	34.2	45.1	7.90
US 73-20	Sumatra No. 2	Nov. 17	466	30.5	43.2	5.96
US 72-204	Pasoeroean	Nov. 22	363	12.1	—	—
US 73-31	SES 2	Nov. 24	654	9.3	—	—
US 73-40	US 56-14-4	Nov. 17	783	9.1	—	—
US 72-241	SES 147B	Oct. 26	515	0	—	—

[1] *All ancestors of the lines are* S. spontaneum.

[2] *Escaped clone found by Dr. G.T.A. Benda, is supposed to be BC1 generation from its morphology.*

TABLE 2-6. Commercial variety

Variety	Date	Grains obs'd	Mat'd grain (%)	Av. size grain (μ)	c.v. grain (%)
CP 57-526	Dec. 16	859	91.4	44.2	6.79
CP 70-401	Nov. 29	470	62.3	47.4	7.84
L 65-69	Nov. 29	486	59.3	47.3	8.61
CP 72-364	Dec. 16	596	50.3	47.7	12.76
L 65-69	Nov. 29	615	46.3	47.3	6.14
L 61-67	Nov. 22	422	42.9	51.4	10.57
CP 72-338	Nov. 26	616	40.1	48.7	8.76
L 62-96	Nov. 29	590	32.4	45.8	9.68
CP 63-365	Dec. 3	460	31.1	49.9	8.23
CP 70-330	Dec. 6	419	26.3	47.5	8.05
CP 70-321	Dec. 1	566	24.6	51.2	14.01
CP 71-334	Nov. 23	333	21.0	47.8	8.67
CP 71-318	Nov. 24	320	17.5	—	—
CP 71-334	Nov. 24	403	13.9	—	—
NCo 310	Nov. 22	748	12.7	—	—
CP 70-330	Nov. 29	471	9.6	—	—
CP 61-39	Dec. 3	364	3.6	—	—
NCo 310	Nov. 29	461	0	—	—
CP 61-37	Dec. 1	118	0	—	—
CP 65-357	Nov. 29	143	0		

Size of pollen grains varied greatly among breeding generations (Table 1). Wild species such as *Ripidium sp.* and *S. spontaneum* have grains with the smallest diameter ranging from 35.4 μ to 40.7 μ and the lowest c.v. The noble variety Fiji 40 had the largest pollen grains and highest c.v. Intermediate generations have grain size and c.v. somewhere between that of the wild species and the noble variety, Fiji 40. Commercial varieties have relatively larger and more variable grains than those of the progressive breeding generations, but not so large as Fiji 40. It was apparent that the c.v. of pollen grains from successive generations increased as grain size increased, with the notable exceptions of US 73-68 (BC_1 *S. spontaneum* SES 147 A) and US 56-15-8 (*S. spontaneum* from Thailand) (Fig. 1). The fact remains that the more highly nobilized the generations, the larger and more variable the pollen grains become.

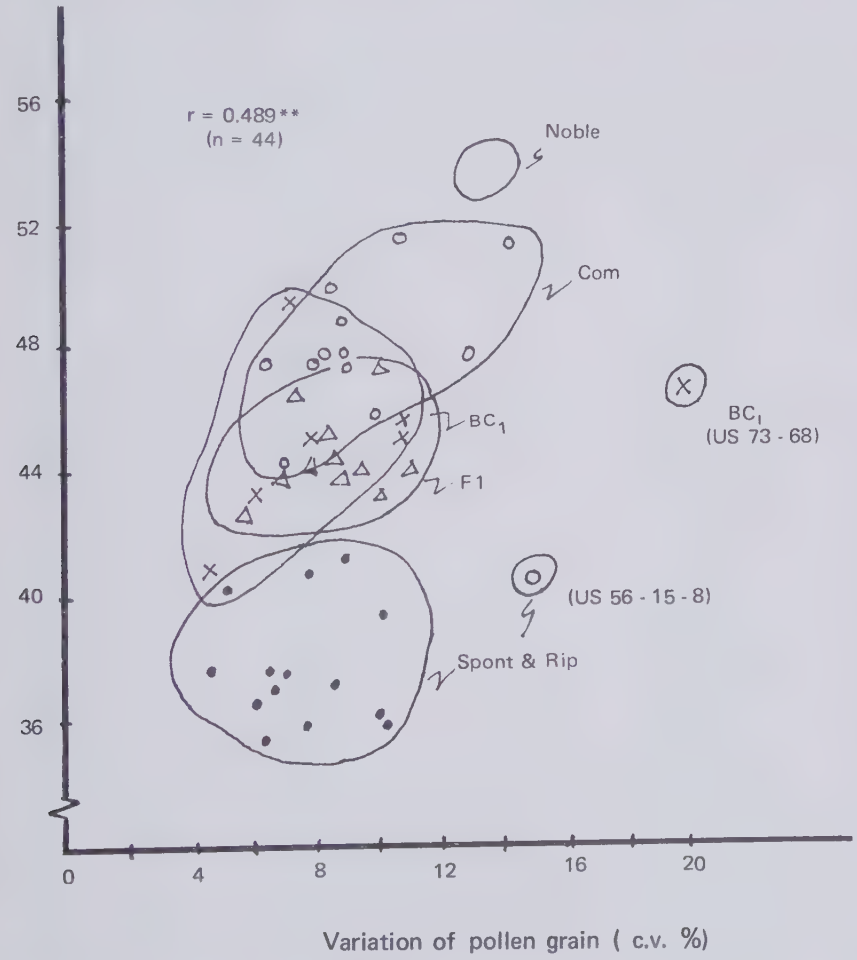


FIGURE 1. Scatter diagram of variation and average size of pollen grains in the breeding generations of sugarcane.

Association between pollen maturity and seed setting: During the 1976 crossing season, 73 bi-parental crosses were made for the purpose of combining the derived features of the commercial and *S. officinarum* L. breeding stocks with the superior germplasm of the wild species (Dunckelman and Nagatomi[11]). Twenty-eight varieties used as males showed broad variation of pollen maturity, while 37 varieties were highly male-sterile.

The association between percentage of mature pollen grains of male parents set in crosses and number of viable seed per gram of fuzz in germination tests are shown in Table 3. More than 400 viable seeds per gram of fuzz were

TABLE 3. Association of the maturity of pollen grains from the male parents and their seed setting.

Rank	Male parent	No. of cross	Maturity of grains (%)	No. of seed set per grm.
1	US 74-103	2	83.0	440
2	US 72-32	2	71.0	426
3	US 73-39	4	92.1	422
4	Benda Sel	3	65.4	199
5	US 56-15-8	2	71.1	195
6	US 73-68	2	61.8	110
7	US 73-28	2	74.3	99
8	L 65-69	6	59.3	97
9	US 74-103	7 (1*)	68.5	78
10	US 72-189	2	66.4	56
11	US 56-15-2	1	64.2	52
12	CP 72-365	7	31.0	47
13	US 72-157	1	34.2	33
14	CP 70-321	2	24.6	25
15	CP 71-334	1	21.0	25
16	CP 70-401	6	62.3	24
17	L 61-67	4	49.2	21
18	US 72-187	1	41.1	16
19	CP 70-330	3	26.3	13
20	L 62-96	2	32.4	12
21	*R. bengalense*	1*	76.5	0
22	US 72-247	1	62.8	0
23	US 72-185	1	48.4	0
24	CP 72-338	1	40.1	0
25	US 72-212	1	30.5	0
26	US 72-214	2	19.7	0
27	US 72-167	1	11.3	0
28	CP 70-330	5	9.6	0

* *marks show the intergeneric crosses.*

obtained from the crosses with US 74-103, US 72-32 and US 72-39 males with a very high percentage of mature pollen grains. Conversely, crosses made with males showing low percentages of mature pollen grains, with a few exceptions, resulted in seed setts ranging from 197-0 per gram of fuzz germ-tested However, intergeneric crosses (*Ripidium sp.* x *Saccharum sp.*) failed to produce any viable seed despite the fact that *Ripidium* had a high percentage of mature pollen grains.

There existed a strong quantitative relationship between the pollen maturity of male parents and average seed production per gram of fuzz from crosses made with the same male parents (Fig. 2). The relationship between both characters may be expressed by the following exponential regression equation 1.

$$W = A \cdot C^{Bx}$$

in which W represents numbers of seed per gram of fuzz; X, the maturity of pollen grains; C, the base of natural logarithms; and A and B, constants. The formula is transformed into logarithms and can be expressed as follows in equation 2:

$$Y = a + bX$$

where Y = log W, log B = b and log A = a.

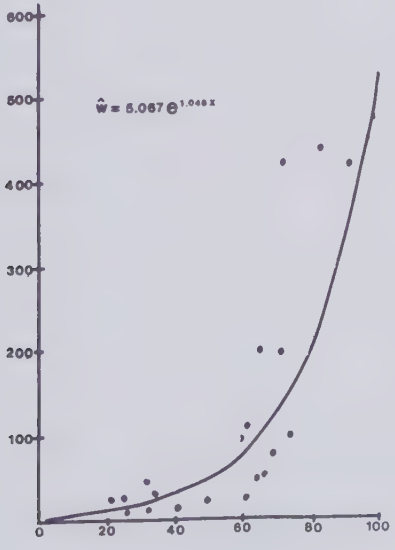


FIGURE 2. Association of the maturity of pollen grains and seed sets per gram fuzz.

Using the available data for the 1976 crossing season, the use of scatter diagrams of seed set per gram of fuzz and maturity of pollen grains (%) resulted in semilogarithmic graph (Fig. 3). In this case, it is found that the association between both characters is essentially straight and highly correlated (r = +0.82) at 1% level of significance. Therefore, the following regression is obtained and also showed significance in the test for linearity.

Equation 3

$$\hat{Y} = 0.020X + 0.075$$

Equation 3 can be further transformed into the exponential formula which corresponds to equation 1:

Equation 4

$$\hat{W} = 5.067e^{1.046X}$$

This formula expressed the growth curve and the exponent value indicates the relative growth rate of seed set (1.046). When pollen maturity of male parents exceeds 60%, seed sets rose sharply.

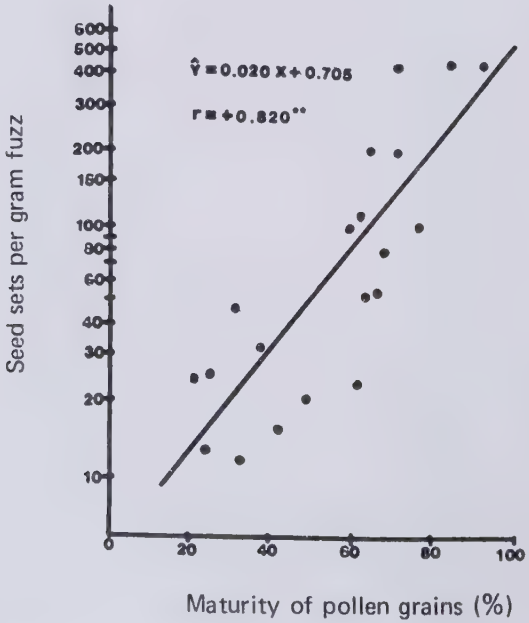


FIGURE 3. Correlation between the maturity of pollen grains and the seed per gram fuzz on logarithmic scale.

Seventy-seven crosses (including four exploratory commercial crosses) made during 1976 were categorized into 11 types of combinations according to each basic breeding generation (Table 4). The estimated seed set for each combination was obtained by substituting the value of each average for maturity of pollen grains into the equation 3 or 4. When *Saccharum* hybrids were crossed with *Ripidum* (72.5% viable pollen), no true seeds were produced; yet according to the formula, there should have been 140 seeds per gram of fuzz produced with the high percentage of mature pollen grains. It was likely that intergeneric sexual incompatibility crosses involving *S. officinarum* and *Saccharum* hybrids, relating lower seed sets resulted when compared with the estimated number. However, commercial hybrids x *S. spontaneum* produced more seeds than the estimate. Generally, excellent seeds sets were obtained from crosses between basic generation hybrids and commercial-type varieties. Abundant seeds were produced from constant amounts of fuzz from crosses with strong male parents, namely commercial x F1 (83% mature pollen grains) and commercial x BC_1 (76.4%).

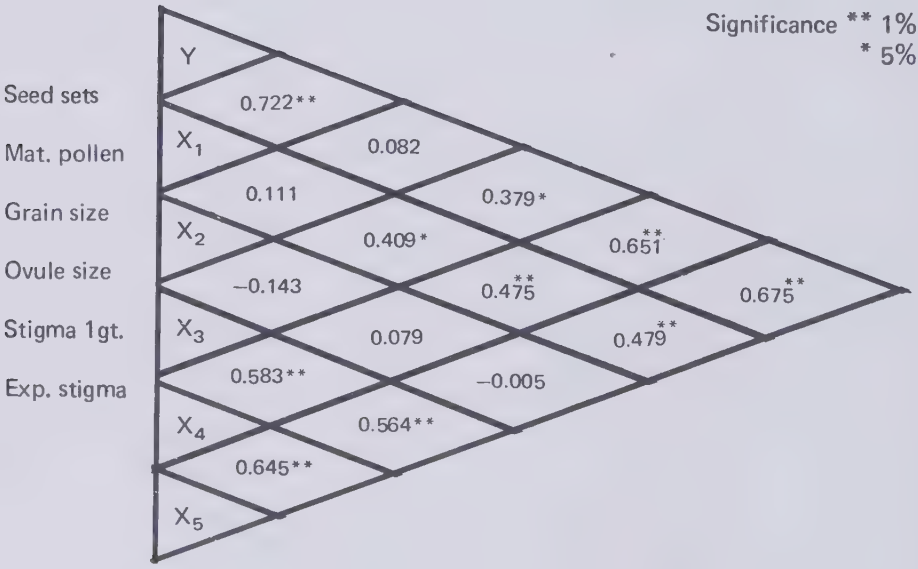
TABLE 4. The association of the types of combination and their maturity of pollen grains and seed sets.

Combination	No. of cross	Av. mat. of grain (%)	Av. seed sets per gram	Estimated seed sets	Difference
Ripidium sp. *	2	72.5	0	140	−140
Noble x hybrid	6	55.6	30	65	− 35
F_1 x F_1	9	55.2	57	64	− 7
F_1 x BC_1	3	34.9	0	25	− 25
F_1 x Com.	28	36.5	36	27	9
BC_1 x BC_1	1	61.8	130	86	+ 44
BC_1 x Com.	3	41.1	72	33	+ 39
Com. x Spont.	2	71.1	195	132	+ 63
Com. x F_1	2	83.0	440	227	+213
Com. X BC_1	12	76.4	211	168	+ 43
Com. x Com.	9	33.8	17	24	− 7
Total/Av.	77	49.6	78	49	% + 29

* *Estimate according to equations (3) or (4) substituting average % of mature pollen grains.*

Correlation of seed sets with characteristics of floral organs: The seed sets of 33 crosses were correlated with five characteristics of the floral organs: pollen maturity (x_1) and size of pollen grains (x_2) of the male parent and ovule size (x_3), length of stigma (x_4) and expanse of stigma exposed outside the glumes of the female parent (Table 5). The characters most highly correlated with seed sets (y) were pollen maturity (x_1) ($r = +0.72^{**}$), expanse of stigma (x_5) ($+0.68^{**}$), length of stigma (x_4) ($+0.65^{**}$) and ovule size (x_3)($+0.38^{*}$). However, size of pollen grains (x_2) was related to none of the characters. Three characters of the pistil (x_3, x_4, x_5) were significantly correlated with each other. In other words, mutual parts of the pistil are proportional. Though there existed some correlations between pollen maturity (x_1) and the pistil parts (x_3, x_4, x_5), the results seemed to be by chance alone.

TABLE 5. Correlation matrix between seed sets and the characters of pollen grains and pistil organ. (N = 33)



Simple correlations between the four selected characters (y, x_1, x_4 and x_5), were positively correlated with each other at 1% level of significance (Table 6). The second order partial correlations between each two characters were calculated by eliminating two other factors. Finally, it was noted that the strongest correlation existed between the seed sets and maturity of pollen ($+0.58^{**}$), as expected, and the second most important correlation was between seed sets and expanse of stigma ($+0.37^{*}$). Therefore, it is evident that better seed sets can be obtained from both higher maturity of pollen grains of male parents and greater expanse of the stigmas of the females.

TABLE 6. Simple and partial correlations between seed set, stigma and maturity of pollen grain.

Correlated characters	Simple correlation[1]	Partial correlation (Eliminated character below)				
		y^2	$x_1{}^2$	$x_4{}^2$	$x_5{}^2$	char's ^{2}
$y : x_1$	0.722**	–	–	0.618**	0.616**	0.582**
$y : x_4$	0.651**	–	0.506**	–	0.383**	0.301
$y : x_5$	0.675**	–	0.543**	0.440*	–	0.371*
$x_1 : x_4$	0.475**	0.097	–	–	0.248	0.017
$x_1 : x_5$	0.478**	−0.017	–	0.256	–	−0.023
$x_4 : x_5$	0.645**	0.367*	0.540**	–	–	0.367*

Character:
y : Seed set per gram.
x_1 : Maturity of pollen grain.
x_4 : Length of stigma.
x_5 : Expanse of stigma.

d.f. [1] *N-2 = 31*
[2] *N-3 = 30*
[3] *N-4 = 29*

Significant level
** *1%*
* *5%*

Elongation of pollen and size of pistil parts: Pollen tube elongation appeared to be associated with pollen size (Table 7). The F_1 variety, US 72-185, showed larger grains and longer pollen tubes than SES 6. The pollen tubes of US 72-185 reached a length of about 1 mm (1093 μ) with the ratio of tube length to grain diameter at 24:1 (Table 7). Conversely, SES 6 showed smaller

TABLE 7. Germination of pollen grain in Moore's media.

Variety	Pollen tube	Size of grain	Tube/ grain
US 72-185	1,250 μ	47 μ	26.6
	1,035	46	22.5
	965	45	21.4
	1,205	46	26.2
	1,010	45	22.4
Average	1,093	45.8	23.8
SES 6	480	38	12.6
	520	38	13.7
	320	37	8.6
	760	38	20.0
Average	520	37.8	13.7

grains with tubes extended to 520 μ or about half the length of the former with a ratio of tube length to grain diameter of 13.7:1. Though the length of pollen tubes between and within varieties was variable, it seemed more proportionate to volume than diameter of grains.

A single flower (spikelet) of sugarcane consists of a gynoecium, an androecium, and accessory parts (Dillewijn[3]), the gynoecium is made up of one pistil ordinarily consisting of a branched style and two feathery stigmas. The androecium consists of one whorl of three stamens, the filaments of which elongate into long threads at the time the spikelet opens. Precise observations were made of pistil parts of parental material under stereo microscopy. Various types of gynoecium are illustrated in Fig. 4. In general the floral organs of *S. spontaneum* are smaller than those of commercial and noble canes. The stigmas of commercial canes are more feathery than those of other canes. The length of the stigma is greater in nobles, smaller in *spontaneums* and intermediate in commercial canes. The style is longest in *spontaneum,* shortest in nobles and intermediate in commercial canes. In the basic breeding generations, the floral organs of the F_1 varieties more closely resemble those of commercial canes than they do *S. spontaneum,* while those of the BC_1 varieties very closely resemble in floral organs of commercial canes. Extra-ordinary numbers of stigmas per spikelet were observed with high frequency in the following clones: CP 70-330 (commercial) showed only one stigma; US 72-192 (F_1), three or four stigmas; US 72-223, four stigmas (Fig. 4).

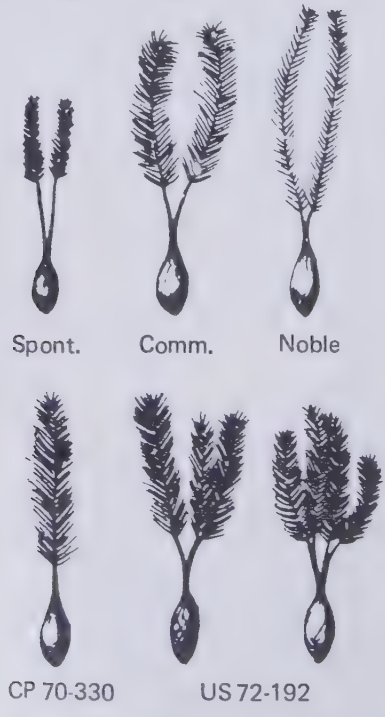


FIGURE 4. The typical types of gynoecium in sugarcane.

When a pollen grain germinates on the stigma, the pollen tube grows into the style and reaches the base of the ovary where an embryo fuses with the male nucleus which has passed through the pollen tube. Therefore, the pollen tube should elongate further than the distance from at least the lowest part of the stigma to the bottom of the ovary in order to accomplish fertilization. The lengths of ovary and style, in *spontaneum,* commercial, and noble cane were 1.32, 1.54 and 1.38 mm, respectively. In a comparison of the elongation of pollen tubes in Moore's media with the average length of pistils of all varieties examined, it was noted that the pollen tubes were shorter than the distances from the lower part of the stigma to the base of the ovary (Fig. 5).

FIGURE 5. Campaign of pollen tube elongation in *vitro* with gynoecium of sugarcane.

Although the breeding material was grown under controlled temperature in the crossing greenhouse, wide differences in pollen maturity were noted (Tables 1, 2). Under such treatment therefore, it is likely that male sterility in many varieties was the result of genetic or cytoplasmic factors (Stevenson[21]). In some instances, male fertility has been recovered as a result of photoperiod treatment (15, 20). Varieties CP 61-37 and CP 61-39, usually male sterile, have been induced to produce 60% and 80%, respectively, mature pollen grains under 12.5 hr photoperiod after floral initiation has taken place (James[15]). If photoperiod treatment can be used as a reliable tool to induce male-fertility in sugarcane, such treatment would be another aid to the crossing work.

When breeding materials are subjected to a natural exposure, the ratio of male sterility to male fertility may fluctuate as a result of variation in temperature patterns through the years. Under conditions at Houma during 1976, about half of the varieties examined, had less than 30% mature pollen grains. Most of the varieties used as females had low mature pollen counts and in dehiscent anthers, a situation which greatly lessens the possibility of selfing. In highly heterozygous plants such as sugarcane, selfing gives rise to low vigor segregates. However, selfs are easily spotted in basic crosses in which hybrid vigor is the rule.

Highly positive correlations, as well as quantitative relationships existed between pollen maturity and seed sets (Fig. 2, 3). The reasons why seed sets increase exponentially over the ratio of pollen maturity is explained by the following observations: 1) in the crossing cubicles, vigorous anther dehiscense occurred in varieties with high pollen maturity and copious amounts of pollen were shed; 2) in varieties with low mature pollen counts, the anthers dehisced poorly or not at all and fell without shedding pollen; 3) positive relationship were noted among pollen maturity, anther dehiscense and shedding of pollen. This evidence is supported by other investigations (Akhtar *et al*[1] and James[15]). Accordingly, it is believed that mature pollen grains were multiplied by increasing qualitative and quantitative rates, thus giving rise to exponential increase of seed sets.

In practical crossing, it is likely that varieties with over 30% mature pollen make good male parents and that those with more than 60% can be expected to produce highly satisfactory seed sets under good conditions. Conversely, the most desirable female parents are those with low pollen maturity (to minimize selfing) and largely exposed stigmatic surfaces (to enhance crossing). A somewhat different view from India (Natarajan *et al*[18]) indicated that the contribution of pollen parents was relatively lower than that of the pistil parents with regard to seed setting in reciprocal crosses. This suggested that poor ovular receptivity took precedence over pollen maturity in some cases. However, in six reciprocal crosses made at Canal Point, Florida (James[15]) no evidence of incompatibility was found, but it was noted that low pollen viability was reflected in low seed sets. The latter condition was found to be true in this study.

It appears that successive increases in size and variation of pollen grains by breeding generations reflect the complexity of genetic backgrounds. For example, the homozygote in the wild species such as *S. spontaneum* and *Ripidium* was relatively similar with stable chromosome numbers. On the other hand, the genetic composition of contemporary commercial varieties is a complexity of several species of *Saccharum,* and the heterozygotes with unstable chromosome numbers may be found even within clones from the same crosses (Stevenson[21]). Those of each breeding generation and intermediate between the parent varieties. Therefore, from the point of view of basic breeding, it may be necessary to focus more attention on the variation of pollen grains, which may be used as an indicator of genetic variability.

Judging from the limited data on pollen germination (Table 7) there existed an association of pollen tube elongation with volume rather than diameter of grains. For example, the ratio of volume of average pollen grains was 1:2.02:2.85 between *S. spontaneum* (38 μ), commercial (48 μ) and noble cane (53.9 μ), respectively. Also, it is likely that wide differences between volume of grains and pollen tube elongation occured between the breeding generations. Therefore, large pollen grains from varieties with highly variable pollen may allow intergeneric crosses with varieties which are usually hard to fertilize because of shortness of pollen tube elongation. With regard to problems and potentials of intergeneric hybridization (Grassl[12]), the great variation in the gametes with the tremendous range in the size of the stainable pollen may explain in part, why some generic crosses are possible.

The pollen tube of the F_1 variety US 72-185, reached a length of 1 mm in Moore's media, which was 20 times the diameter of the pollen grains (Table 7). These results are in general agreement with Moore and Jung's[16]. However, it was found that the pollen tubes of US 72-185 were still too short to accomplish fertilization in crosses at Houma (Fig. 5). It is surmised that elongation of the pollen tube *in vivo* may be more enhanced with a supply of certain nutrients and accelerative substances not found in artificial media. Since the average length of the stigma is about 2 mm, or twice the length of the pollen tube in media, it is likely that the position of the pollen grain on the stigma has an effect on the accomplishment of fertilization. Consequently, a large expanse of stigma outside the glume is a factor contributing to increased setting of seeds because of greater exposure with reference to the ovary and to the lodging of pollen grains on the stigmatic surface.

Investigations on the effect of various concentrations of nutrients in media (Moore and Jung[16]) indicated that pollen grain germination showed a much broader optimum than growth of pollen tubes and that *S. officinarum* and *S. spontaneum* differed in nutrient requirements. This may explain the difficulty in obtaining successful seed set in crosses between different genera with different nutrient levels found in stigmatic fluid.

In interspecific crosses (Table 4), noble x hybrid combinations resulted in relatively low seed sets. The probable reason is that the noble variety, Fiji 40, was subjected to low temperature in the unheated photoperiod house and this adversely affected ovular fertility (Brett[3]). This does not infer that all interspecific crosses involving commercial canes x *S. spontaneum* produced a profusion of viable seeds. In many canes, seeds from interspecific crosses involving *Saccharum* are relatively easy to attain and the success of such crosses is governed mainly by the maturity of the pollen grains of available breeding material.

Intergeneric crosses between *Ripidium* and *Saccharum* hybrids were unsuccessful during 1976. In 1974, four crosses were made with *Erianthus arundinaceous* and one with *E. procerus* as non-recurrent parents and *Saccharum* hybrids as recurrent parents. Only the latter cross was successful (Dunckelman and Blanchard[10]). It is thought that these intergeneric crosses were governed

by certain incompatibilities between the genera rather than by the characteristics of the floral organs. It is conceivable that the characteristics of different genera may be more easily combined by the use of bridging germplasm that is closely related to both wild genera and *Saccharum* species.

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LAS CARACTERISTICAS DE POLEN Y PISTILO EN RELACION CON EL DESARROLLO DE LA SEMILLA BOTANICA EN CRUZAMIENTO DE CAÑA DE AZUCAR.

S. Nagatomi y P. H. Dunckelman

RESUMEN

El presente estudio fue realizado en Houma, Louisiana, con el objetivo de investigar la asociacion entre el desarrollo de la semilla verdadera y caracteristicas de los organos florales en cruzamientos de caña de azucar.

Se encontro una correlacion positiva entre generacion de cruza y tamaño y variabilidad de los granos de polen. A medida que la generacion implicaba una mayor nobilizacion, los granos de polen resultaban de mayor tamaño y mas variables.

El desarrollo de la semilla se incremento en forma exponencial en relacion con la madurez del polen de los machos, y estuvo fuertemente asociada con la dehiscencia de las anteras y el derramado de los granos.

Los tubos polinicos de los granos de polen germinados en medios artificiales aparecian demasiados cortos para cumplimentar la fertilizacion, en comparacion con las partes del pistilo.

Pueden esperarse mejores desarrollos de la semilla en cruzas interspecificas cuando el porciento de polen maduro en los machos excede el 60%, y los estigmas en las hembras se encuentran totalmente expandidos fuera de las glumas.

El desarrollo de las semillas en cruzas intergenericas parecio estar gobernado por incompatibilidad sexual entre los padres, mas que por caracteristicas especiales en los organos florales. Se discuten algunas posibilidades para facilitar las cruzas intergenericas.

FERTILITY, SEED STORAGE AND SEED VIABILITY IN SUGARCANE

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ABSTRACT

Analysis of seed from four open pollinated hermaphrodite clones and two biparental crosses showed an average of 2,013 florets per gram fuzz and seed set between 3.1 and 22.7%. Seed stored at 28°C and 90% RH lost 90% of viability within 80 days while seed stored for same period with silica gel at 28°C and 90% RH and without silica gel at 10°C and 65% RH retained most of the initial viability. Seed dried to 10.4% moisture and stored with silica gel in high density polythene bags at -10°C in a freezer maintained germinability beyond two years. The significance of low fertility, short and long term storage of fuzz in a breeding program are discussed.

INTRODUCTION

To the extent that modern sugarcane breeding program succeed in producing large populations of seedlings, sugarcane appears superficially to have high sexual fertility and seed viability. However, the norm in all breeding programs seems to be a rather low rate of seed set: for example, biparental crosses which produce few or no seedlings may account for 30% of the annual effort. As a result of many studies pollen production and fertility are quite well understood but only one quantitative record of seed fertility has been found in literatures (Price[3]).

Seed storage and viability are of added importance now that breeders are planting population samples of an optimum size for family evaluation, are processing fuzz (Silva[5]) and are building up seed banks under some form of cold storage for future evaluation. McIntosh[2] working with 12 crosses found fuzz stored for seven months near freezing temperature with calcium chloride and carbondioxide lost 40% of its initial germination potential.

This report summarizes some experimental work aimed at helping to establish a system of seed production, storage and germination for a commercially-oriented breeding program.

MATERIALS AND METHODS

Fuzz Composition

Open pollinated fuzz from four hermaphrodite parents and fuzz from two biparental crosses was collected at maturity and dried with a fan heater (35°C 60% RH) for 40h. Total florets in duplicate one gram samples of fuzz were counted. From each sample 200 florets were dissected and seed number and condition noted. Duplicate 0.5g samples of fuzz were sown in a standard sterilized compost maintained at 35-40°C day/30°C night for 10 days and seedling emergence recorded.

Moisture content of fuzz was determined in two crosses at harvest, after drying with a fan heater, and after packing with silica gel by noting the weight after further drying for 24h at 100°C.

Results

Data from dissections and germination tests are given in Table 1. The naked seed weighed 0.54 ± 0.05 mg and measured 1.5 ± 0.03 mm long x 0.64 ± 0.005 mm broad. In some crosses seed was of uniform size while in others up to 30% were smaller or shrivelled, but most of these germinated. Much of the volume of fuzz is accounted by rachii, glumes, hairs, stigmas and anthers.

TABLE 1. Florets, seed and seedlings in 1 gram fuzz

Clone/cross	Florets	Seeds	Seedlings	Seed set %
B 70607 OP	1924 ± 2	436 ± 20	389 ± 19	22.7 ± 1.1
B 73486 OP	1703 ± 38	277 ± 20	245 ± 25	16.3 ± 1.5
Q 83 (x B 63119)	2151 ± 39	215 ± 8	107 ± 29	10.0 ± 0.6
B 73405 (x B 71395)	1786 ± 0	167 ± 23	142 ± 12	9.4 ± 1.3
B 73378 OP	2272 ± 14	71 ± 7	79 ± 3	3.1 ± 0.4
B NCo 310 OP	1854 ± 10	57 ± 4	65 ± 5	3.1 ± 0.3
Mean	2013	204	171	10.8

Taking the average from Table 1 of 2,013 florets per gram fuzz and an average weight of fuzz per inflorescence of 12.2g (the average for 1978 crossing program) it can be estimated that on an average an inflorescence contained about 24,600 florets. From routine germination tests the best polycross in 1978 gave 730 seedlings per gram, or about 8,900 seedlings per inflorescence, a fertility of less than 37% while quite a few crosses were sterile.

Warm air drying brought the moisture content of fuzz, expressed on wet weight basis, from 12% to 10.4% and silica gel did not reduce this further. As shown in the storage experiments, however, drying below 10.4% level is not necessary for maintaining viability in storage for a few years.

Seed Storage

In the first experiment fuzz from two open-pollinated parents was dried in warm air and sealed in polythene bags and stored at room temperature (28°C 90% RH) with and without self-indicating sillica gel or in a domestic refrigerator (10°C 65% RH). Half gram samples were test germinated at 10-day intervals up to 80 days.

In the second experiment, fuzz from four parents was packed with silica gel in high-density polythene bags in a freezer (-10°C) and duplicate germination tests made at intervals of 6 months over 24-month period.

In the third experiment, fuzz from two parents was stored with silica gel under two temperature regimes – refrigerator and freezer and duplicate germination tests made at intervals of six months over a 24-month period.

Results

In Experiment 1 (Table 2) fuzz germinability dropped steadily at room temperature without silica gel, but showed much smaller drop without silica gel under refrigerated conditions ($P = 0.01$-0.001) There was no significant drop in the germinability of fuzz stored with silica gel at room temperature. However, to maintain dry conditions it was necessary to replace the silica gel three times in the 80 days at room temperature.

TABLE 2. Storage conditions and mean fuzz viability – percent initial germination (seedlings/g fuzz)

Storage	Days of storage								
Conditions	0	10	20	30	40	50	60	70	80
28°C	100 (113)	79	48	32	32	38	15	10	10
10°C	100 (91)	100	100	100	93	84	89	77	86
28°C with silica gel	100 (91)	93	85	77	65	83	97	98	74

In Experiment 2 (Table 3) the drop in germination under freezer storage with silica gel over two years was not statistically significant and there was no parent x storage period interaction.

TABLE 3. Viability in stored fuzz over two years percent initial germination (seedlings/g fuzz)

	Months of storage				
Clone	0	6	12	18	24
B 70607 OP	100 (389)	97	98	92	87
B 73486 OP	100 (245)	100	69	89	93
B 73378 OP	100 (79)	110	85	72	99
NCo 310 OP	100 (65)	105	120	112	110
Mean	100 (195)	100	89	91	92

In Experiment 3 (Table 4) there was no significant difference between the two cool storage regimes, but the silica gel required changing at least twice during the 2-year storage in refrigerator, while it remained active under freezer conditions.

TABLE 4. Storage temperature and mean fuzz viability — percent initial germination (seedlings/g fuzz)

Temperature	Months of storage					
	0	6	12	18	24	0
-10°C	100 (162)	103	73	90	85	
19°C	100 (162)	101	76	82	94	10°C

DISCUSSION

Seed set in six crosses studied is low. In our breeding program there are three main types of crosses

(a) genetic base-broadening crosses, mostly utilizing nobles as female parents; 5

(b) biparental crosses between commercial hybrids normally using male-sterile female parents; 132 and

(c) polycrosses made in racks in the open air. 115

For 1978 season in these three main types of crosses germination was 5,132 and 115 seedlings per gram fuzz and seed set was 0.2, 1.6 and 5.7%, respectively. The very low seed set rate of the base-broadening crosses may be due to some genetic sterility since in most cases these are crosses between two species or backcrosses of such hybrids on to nobles. However, Price[3] reported a low percentage fertility in open-pollinated nobles. A further factor may be the greater sensitivity of nobles to the artificial environment used in making crosses. Among commercial hybrids aneuploidy is common so that meiotic irregularities may be common and in some case could cause sterility.

Viability of fuzz dried to 10.4% moisture content was fully maintained for over two years storage below freezing point. Longevity is prolonged by decreasing moisture content and lowering temperature in orthodox seed (Roberts[4]) Seeds of most species kept in equilibirum with an RH of 20-25% below 0°C maintain capacity to germinate for hundreds of years (Harrington[1]) Since sugarcane behaves like an orthodox seed, with further drying by other desiccation methods there is every possibility of storage at least for several decades. Long-term storage of fuzz would be quite valuable for germplasm conservation since a large amount of viability could be stored economically by comparison with living clonal collections in the field.

For shorter term storage, viability can be maintained by packing fuzz with silica gel in properly sealed containers, kept at room temperatures. In our experience high density polythene bags, properly heat-sealed, and a ratio of 1 part silica gel; 2 parts fuzz by weight kept in freezer, provide adequate means of short term (± 4 years) storage.

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FERTILIDAD, CONSERVACION Y VIABILIDAD DE SEMILLA DE CAÑA DE AZUCAR

P. Seshagiri Rao

RESUMEN

Analisis de la semilla obtenida de cuatro clones hermafroditas en polenizacion libre y de dos cruzas biparentales, mostró un promedio de 2.013 capsulas por gramo de "fuzz" (semilla sucia), con un promedio de formacion de semilla entre 3.1 y 22.7 por ciento. La semilla conservada a 28°C y 90% HR perdio el 90% de la viabilidad dentro de los 80 dias, mientras que semilla conservada durante el mismo periodo con silica-gel a 28°C y 90% HR y sin silica-gel a 10°C y 65% HR, retuvo la mayor parte de la viabilidad inicial. La semilla desecada a 10.4% de humedad y conservada con silica-gel en bolsas de polietileno de alta densidad a -10°C en una congeladora, mantuvo su capacidad de germinacion mas de 2 años. Se discute la significacion de la baja fertilidad y de la conservacion a coto y largo plazo de la semilla para un programa de mejoramiento.

IMPROVED GROWTH OF SUGAR CANE SEEDLINGS IN SOIL MIXTURES CONTAINING COCONUT FIBER WASTE

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ABSTRACT

Two greenhouse experiments conducted in Trinidad showed that better growth of sugar cane seedlings was obtained when coconut fiber waste was added to the soil mixture in the flats. The improvement was attributed to better physical properties, drainage and aeration of the mixture containing coconut fiber waste. This mixture remained uncompacted, spongy and friable in the seedling flats after daily watering for six weeks. Analyses of soil mixtures with and without coconut fiber waste showed increases in porosity and reduction in bulk density when the fiber waste was used in soil mixtures. The use of coconut fiber waste as a soil amendment is recommended wherever easily compacted or crusted soils are used for the culture of sugar cane seedlings.

INTRODUCTION

Coconut fiber waste (CFW) is granular, spongy, organic substance – a byproduct obtained after the removal of the fiber from coconut husks. The material is known in Trinidad and Tobago as coconut fiber bast. Evans[3] reported that CFW was used at I.C.T.A., Trinidad, in propagating bins for the rooting of cacao cuttings. Vlitos and Davies[5] stated that CFW, as a medium for growing plants, not only provides excellent drainage conditions but is also sufficiently hygroscopic not to require the recirculation of water and nutrient solutions as is necessary in conventional hydroponics systems. Trials using CFW as a soil conditioner in sugar cane seedlings flats were started in November 1977, in order to improve the germination and growth of seedlings to be tested yearly at Caroni Research Station. It was necessary to expand the variety selection program following the outbreak of smut disease (*Ustilago scitaminea*, Sydow.) in Trinidad in 1976. Observations of poor seedling growth in the flats suggested that the soil mixture was unsatisfactory. Based on germination tests done at the West Indies Central Sugarcane Breeding Station, it was concluded that up to 30% of the seedlings were being lost in the flats or after transplanting into polythene bags. Seedlings death in the flats was thought to be due mainly to surface crusting and compaction of the soil mixture in the flats, with resulting poor water relations and insufficient aeration in the root zone.

The purpose of the investigation therefore, was to discover whether the addition of CFW to the soil mixture would improve the germination and growth of the sugar cane seedlings. A second objective was to assess the relative merits of soil mixture sterilization using heat as compared to fumigation with methyl bromide. Physical properties of the two mixtures were measured in order to compare the physical characteristics of the mixtures. Determinations of soil nutrients were also made for the mixture containing CFW.

MATERIALS AND METHODS

Two experiments were planted in November 1977 in the greenhouse at Caroni Research Station, which is situated at sea level in Trinidad. Temperatures in the greenhouse ranged between 20°C and 31°C. Relative humidity in the daytime fell between 40% and 50%, but rose to 92-98% at night.

Metal flats measuring 50 cm x 35 cm x 8 cm were used to contain the soil mixtures for both experiments. The flats were washed and painted with black bituminous paint two weeks before the trials were started. A layer of clean broken clay tiles, 1 to 2 cm deep, was evenly placed at the perforated bottom of each flat to provide good drainage of excess water. The soil mixture was prepared by composting equal parts by volume of stale filter press mud (about 1 year old) and a fine sandy loam soil (River Estate series). This compost mixture was prepared four months before the trials were begun, and to this compost, coarse graded sand, 2 to 3 mm in diameter, was added in a ratio of 2 parts soil compost to 1 part coarse sand. The sand was carefully mixed with the compost using shovels and a soil shredding machine until the sand appeareed to be homogeneously distributed in the mixture. This mixture was used in the flats as the treatment without CFW. The treatment with CFW was prepared by adding CFW to the above, so that the CFW comprised 50% by volume.

In Experiment 1 the soil mixtures were not sterilized before planting the seeds. In Experiment 2 heat sterilization was compared to no sterilization and to fumigation using methyl bromide (Dowfume) at the recommended rate. Three days were allowed between heat treatment and planting the seeds, and ten days were allowed after fumigation by methyl bromide. Prior experiments with sugar cane seedlings have established these periods as safe for heat and methyl bromide treatments respectively.

Each flat in both experiments was sown with 1 gram of seed taken from the same high germinating cross. After sowing the seeds, a solution of Benlate fungicide was applied at 0.25 g per flat using a fine spray. Watering was done carefully once or twice daily from planting until the experiment was completed. The water was taken from the potable water supply in order to ensure that it would be as free from bacterial and fungal contamination as possible.

Experiment 1 was sown on the 4th of November, 1977 and measurements of seedling growth were taken 42 days after sowing. In this experiment the two soil mixtures were compared using 2 flats of each mixture. Ten seedlings were taken at random from each flat and the longest roots and leaves of each seedling were measured and recorded. Fresh weights and dry weights of the seedlings were also recorded.

Experiment 2 was sown on the 16th of November, 1977 and consisted of two soil mixture treatments (with and without CFW) factorially combined with three sterilization treatments to give a total of six treatments. Each treatment was replicated twice so that 12 flats were used for this experiment. Germination counts were taken 10 days after planting, and seedling growth measurements and weights were taken on 10 plants per flat at 26 days after sowing using the same procedure as for Experiment 1. The data of both experiments were subjected to analysis of variance, and means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

In Experiment 1, the soil mixture containing CFW gave larger and stronger seedlings at 42 days after planting as shown in Table 1. Significant differences in fresh weight and dry weight of seedlings were found in favor of the mixture containing CFW. Careful visual observation of the soil mixtures showed that the mixture containing CFW remained uncompacted, spongy and friable in the seedling flats after daily watering for six weeks. Water applied to the surface was rapidly absorbed by the mixture containing CFW. Six weeks after planting the mixture without CFW appeared to have become dense and compact, and with crusting of the surface which would reduce aeration and which impeded percolation of water into the soil mixture. Crusting of the soil surface is characteristic of the River Estate soil as reported by Ahmad and Roblin[1]. The composting with filter press mud did not prevent the formation of the surface crust under the conditions of the experiments.

TABLE 1. Experiment 1 — Comparison of Sugar Cane Seedling Growth at 42 Days in Soil Mixtures with and without Coconut Fiber Waste

	Soil mixtures	
	+ CFW	— CFW
Root length (cm)	18.3 a	14.7 a
Leaf length (cm)	52.5 a	33.2 a
Fresh weight of 10 plants (g)	16.9 a	7.5 b
Dry weight of 10 plants (g)	2.4 a	1.2 b

Values in rows with a common letter are not significantly different at the 5% level.
Coefficients of variation: Root length 8.8%, Leaf length 10.0%, Fresh weight 1.0%, Dry weight 2.8%.

As was shown in Table 1, seedling growth was poor by comparison with mixture containing CFW, and in addition 10% or more of the seedlings turned brown and died before 42 days. When the seedlings were removed for transplanting into polythene bags, it was found that many of them have poorly developed root systems. Such seedlings often do not survive the shock of transplanting.

The results of Experiment 1 were confirmed by the data taken in Experiment 2. In this second experiment, measurements and weights of seedlings were taken at 26 days rather than at 42 days as was the case in Experiment 1. In spite of this, significant differences between soil mixtures were found when root and leaf lengths were measured (Table 2). Numbers of seedlings germinated at 10 days after planting were significantly lower in the mixture without CFW which had formed a surface crust after watering for a few days. Ahmad and Roblin[1] also reported reductions in seedling emergence due to crusting of River Estate soil.

TABLE 2 Experiment 2 – Effect of Soil Mixtures and Sterilization Treatment on Germination and Growth (at 26 Days) of Sugar Cane Seedlings

	Soil mixtures		Sterilization treatments		
	+ CFW	– CFW	M. bromide	Heat	Unsterilized
Germination count at 10 days	193.8 a	145.3 b	162.0 a	175.8 a	171.0 a
Root length (cm)	8.5 a	6.1 b	5.8 a	7.7 a	8.5 a
Leaf length (cm)	21.1 a	14.1 b	15.7 a	20.8 a	16.3 a
Fresh weight of 10 plants (g)	2.2 a	1.5 a	1.5 a	2.3 a	1.7 a
Dry weight of 10 plants (g)	0.29 a	0.24 a	0.23 a	0.32 a	0.25 a

Values with a common letter in rows within columns are not significantly different at the 5% level.

Coefficients of variation – Germination count 9.5%, Root length 11.1%, Leaf length 15.4%, Fresh weight 29.1% and Dry Weight 16.7%.

Differences in fresh weight and dry weight of seedlings were present but are not statistically significant, probably due to the small sample size of 10 plants per flat, and the early period of assessment at 26 days planting. There were no significant differences, measured in seedling germination and growth, between the soil mixture sterilization treatments (Table 2).

It has previously been reported by Donelan[2] that addition of CFW as a soil conditioner caused the soil mixture to become less dense and with much improved aeration and drainage. Vlitos and Davies[5] successfully used a growing medium consisting of 100% CFW plus mineral nutrients for cultivating a wide range of vegetable crops, and reported that the medium provided excellent drainage conditions and was also sufficiently hygroscopic for their purposes. Matkin *et al.*[4] stated that container soils consisting of a mixture of fine and coarse sands with relatively small amounts of silt and clay, can become very compact and thus poorly

TABLE 3. Comparison of Physical Characteristics of Soil Mixtures with and without Coconut Fiber Waste.

	Soil mixtures	
	+ CFW	– CFW
Organic matter content	9.8%	3.6%
Particle size distribution		
Coarse sand	46 %	52 %
Fine sand	26 %	22
Silt	16	15
Clay	12	11
Bulk density (g per cm^3)	0.8	1.4
Total porosity (% volume)	62 %	51 %
Macro porosity (% volume)	8.0%	6.6%
Water retention		
at 1/3 Bar (field capacity)	24.5%	19.0%
at 15 Bar (permanent wilting)	18.6%	10.6%
Available water capacity	5.9%	8.4%

aerated. As shown in Table 3, the soil mixture without CFW fits this description and had a high bulk density of 1.4 g per cubic cm. The addition of 50% by volume of CFW reduced the bulk density of the mixture to 0.8 g per cubic cm, and improved the porosity of the mixture. Total porosity increased by more than 20%, and macroporosity increased by a similar amount. Addition of CFW therefore caused a general increase in pores of all sizes and thereby improved both soil aeration and soil water retention (Table 3). The figures, however, show that there was less available moisture in the mixture containing CFW. The superior plant growth in this latter mixture indicated that the available moisture supply was adequate, and

suggests that aeration in the root zone was an important factor in causing the seedling growth differences which were found in both experiments.

Observations of the mixtures in the greenhouse showed that the upper layer of the mixture without CFW dried out between applications of irrigation water at 24 hour intervals and formed a surface crust, whereas the mixture with CFW remained moist and so provided better conditions for seed germination and seedling growth.

CFW is a largely inert organic material which is also highly resistant to decomposition and probably does not contribute significantly to the nutrition of sugar cane seedlings. Analysis of CFW showed levels of 0.73% N, 0.05% P and 0.47% K on a dry matter basis. The N level or 0.73% dry matter does not imply that this nitrogen is available to plants, but is merely a reflection of the organic nature of CFW. Vlitos and Davies[5] found that CFW alone was unable to support the growth of vegetable crops.

Table 4 shows that high levels of N, P, and K were found in the soil mixture with CFW. Much of these nutrients can be attributed to the filter press mud which was present at 20% by volume in the mixture. In these experiments it has been found that the soil mixture with CFW adequately supported up to 200 sugar cane seedlings per flat for a period of 6 weeks without addition of N, P or K fertilizers.

TABLE 4. Chemical Properties of the Soil Mixture Containing Coconut Fiber Waste

Per cent organic matter	9.8
Per cent carbon	5.7
pH	5.9
Total nitrogen	0.5%
Phosphorus, pp,	1360
Potassium, ppm	790

Note: Analyses were conducted on the 0 – 2 mm soil fraction.

CONCLUSION

The use of coconut fiber waste, as a soil amendment, can be recommended wherever easily compacted or crusted soils are used for the culture of sugar cane seedlings. The material is inexpensive, light in weight, easy to transport and to use in preparing soil mixtures. It is also available in most tropical countries in which sugar cane is grown.

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MEJOR CRECIMIENTO DE PLANTINES DE CAÑA DE AZUCAR EN MEZCLAS DE SUELO QUE CONTIENEN RESTOS DE FIBRA DE COCO.

A. F.Donelan

RESUMEN

Dos experimentos conducidos en Trinidad bajo condiciones de invernaculo muestran un mejor crecimiento de plantines de caña de azucar, cuando se agrega a la mezcla de suelo los restos de fibra de coco. La mezcla se mantiene sin compactacion, esponjosa y friable en las bandejas con plantines, despues de riegos diarios por seis semanas. Los analisis de las muestras de suelos con y sin restos de fibra de coco, muestran incremento en la porosidad y reduccion en la densidad en el primer caso. Se recomienda en consecuencia el uso de este aditamento en el suelo, especialmente cuando se utiliza suelo de facil compactacion para la mezcla en la germinacion de plantines.

Aun no estan disponibles los resultados de los rendimientos de los retonos aunque los primeros resultados de crecimiento indican que NCo 293 y NCo 376 son practicamente tolerantes a diuron + Sencor. Diuron + Velpar es igualmente o ligeramente menos fitotoxico que

diuron + 2,4-D + S en todas las variedades. De nuevo la variedad N 8 es la mas susceptible ya que su crecimiento es severamente retardado por los tres tratamientos. El patron y grado de fitotoxicidad debido a diuron + 2,4-D + S en el retoño es muy similar al que se manifiesta en la plantilla aunque la dosis que se le aplicó al retoño era de solamente la mitad de lo que se le aplicó a la plantilla; el retoño tambien era mas joven al momento de la aplicacion.

EFFECT OF VENTILATION ON GERMINATION AND VIGOR OF SUGARCANE CARYOPSES

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ABSTRACT

Caryopses of 5 sugarcane cultivars were cleaned with seed-blower to remove physical impurities with a ventilation strength of zero to 9.6 mg/cm². In the best cleaning treatment, around 56% of the impurities were eliminated reducing the initial caryopses weight by more than 63%.

Germination percentage and vigor index of cleaned caryopses in ideal conditions were higher than untreated ones.

INTRODUCTION

The use of caryopses for sugarcane propagation is restricted to breeding program. There is only a few reports about seed pathology (Loveless & Smith[6], Byther & Steiner[2,3] and Sanguino[9]). Studies about seed cleaning process are restricted to Silva[10].

Reports relating weight and purity effects on germination, vigor and productivity are common in field crops as corn, wheat, oat, barley and rice (Bleasdale[1], Cicero[4], Ferraz[5] and Marcos *et al*[7]).

It is a well known fact that in all field crops heavier seeds show greater percentage of germination, more vigorous seedlings and higher stand than lighter ones. According to Bleasdale[1], the greater and heavier seeds have higher probabilities of being viable. Thus small seeds could be eliminated by screening, selection or ventilation treatments to improve yields.

During the development of an early screening method for smut resistance in sugarcane seedling, the necessity of working with purified sugarcane seed was found imperative. The effect of pre-cleaning and cleaning of sugarcane caryopses were studied by measuring germination and seedling vigor of cleaned seeds.

MATERIALS AND METHODS

Caryopses produced in the Flowering and Breeding Station of Serra do Ouro, belonging to the National Sugarcane Improvement Program (PLANALSUCAR) were pre-cleaned by Silva's[10] method to defuzz sugarcane caryopses.

Pre-cleaned caryopses with approximately 7% moisture content were cleaned with a seed-blower machine described by Musil[8], E.L. Ericson Products, Model B, series 333 made in Borckings, S. Dakota, in the Department of Seed Technology of the College of Agriculture ESALQ, USP, Piracicaba, S.P.

In the first experiment, five sugarcane cultivars were utilized: Co 62-175, F 154, NCo 339, PR 1117 and RB 68121. Each cultivar was divided into 6 lots and each of them was subdivided into 5 sublots of 1.5 gram of uncleaned caryopses. The sublots were ventilated during 2 minutes with the blower valve opening set to produce ventilation strength varying from zero to 9.9 mg/cm^2.

After blowing, the light caryopses and impurities were separated from the heavier caryopses and impurities in the blower tube. The heavier and light seed plus impurities were weighed and converted into percentage.

The experiment had 6 ventilations strength, 5 cultivars and 5 replications for each ventilation strength.

In the second and third experiments, the same cultivars were used. Caryopses were ventilated on the seed-blowers with ventilation strength varying from zero to 9.9 mg/cm^2. The heavier caryopses were disinfected for two hours with zinc omadine solution (zinc-2-pyridimethiol-1-oxide) with 1440 ppm of active ingredient.

The treated caryopses were seeded in aluminum trays with 5 x 1x30 cm, containing 400 ml of sterilized water-agar (W.A.) substratum. After sowing, the trays were covered with glass plate, and incubated for 7 days at 29^{o}C under continuous light from 9 fluorescent daylight, 40-watt bulb set at distance of 1 meter.

In the second and third test the caryopses were used to determine the ventilation effect on germination and seedling vigor respectively.

The germination evaluation was made in the 7th day considering germinated seed as those with radicle and plumule regularly developed. The vigor index of caryopses was determined by daily counting of germinated caryopses of each parcel during the period of seven days, according to Marcos Filho *et al*[7]. This method follows the principle that caryopses which germinate more rapidly are the most vigorous.

In both experiments the experimental design was completely randomized with 6 treatments, 4 replications and each plot had 100 seeds.

RESULTS AND DISCUSSION

The elimination of impurities increases with ventilation strength, as shown on Table I. The variance of removed impurities percentage must be partly attributed to the different caryopses medium weight of each cultivar.

It is useful to utilize a ventilation strength which presents a little variance rate in order to standardize the valve opening of the blower.

This choice should be done assuming the criterion not to eliminate in excess heavier caryopses.

The caryopses germination and vigor (Tables 2 and 3) increased with the increase of ventilation strength providing a greater number of seedlings in each cleaned plot.

At the ventilation strength of 9.6 mg/cm^2, the average germination of all cultivars statistically differed from the control plot.

From the results presented on Table 3 one can verify that the caryopses vigor was higher in cleaned caryopses than the control plot. The best vigor index were obtained with ventilation of 9.6, 9.8 and 9.9 mg/cm^2. There were no statistical differences among the 3 averages.

The separation of caryopses into 2 blocks, one lighter and other heavier, as

TABLE 1. Impurities, steriles and light caryopses eliminated during the ventilation of 5 sugarcane cultivars.

Ventilation Strength mg/cm^2	Percentage of Impurities eliminated in 5 cultivars					General Mean %	V. R.
	CO 62-175	F 154	NCD 339	PR 1117	RM 68121		
0.0	00.0	0.0	0.0	0.0	0.0	0.0	0.0
8.0	36.1*	29.4	56.2	17.4	47.0	37.2	40.54
9.5	45.3	44.4	71.3	33.61	52.0	49.3	28.26
9.6	49.2	55.5	76.5	40.9	58.0	56.0	23.57
9.8	57.4	70.0	86.0	59.9	60.0	66.0	17.60
9.9	63.2	80.4	88.8	67.1	64.3	72.7	15.51

V. R. = Variation rate

* *Each figure represents mean of 5 replications of 100 caryopses per plot.*

TABLE 2. Ventilation effect on germination of sugarcane caryopses.

Ventilation strength mg/cm²	Mean % of germinated caryopses of 5 cultivars					
	CO 62–175	F 154	NCD 339	PR 1117	RB 68121	General mean
0.0	36.50 a	20.00 a	1.75 b	32.00 a	21.75 a	22
8.7	65.00 b	28.25 ab	12.00 b	35.00 ab	69.25 b	41
9.5	66.25 bc	31.50 abc	11.75 bc	41.00 bc	79.50 bc	46
9.6	72.00 bcd	38.75 bcd	23.50 d	43.50 c	78.50 bc	51
9.8	69.00 bcd	47.25 d	24.50 d	39.50 bc	84.25 c	52
9.9	78.75 bcd	43.50 d	14.50 bcd	41.50 bc	78.50 bc	51
V. R of the transformed data	16.15	17.64	37.72	7.88	44.66	

* *The data were transformed into arcsin for the statistical analysis, and in each cultivar means with the same letter are statistically identified by Tukey's test at 5% level.*

TABLE 3. Ventilation effect on sugarcane caryopses vigor.

Ventilation strength mg/cm²	Vigor index of 5 cultivars					
	CO 62–175	F 154	NCD 339	PR 1117	RB 68121	General average %
0.0	12.41 a*	6.20 a	0.41 a	8.99 a	8.10 a	7
8.7	21.45 b	8.52 ab	3.16 b	9.95 a	27.42 b	14
9.5	20.71 bc	9.43 bc	2.54 b	12.11 b	32.39 bc	15
9.6	25.90 d	11.42 bcd	6.10 c	12.38 b	32.68 bc	17
9.8	23.08 bcd	14.12 d	5.86 c	11.43 ab	35.17 c	17
9.9	26.42 d	12.96 d	3.46 b	11.54 b	33.71 c	17
V. R of the transformed data	13.16	15.59	36.59	7.58	22.70	

* *The mean of vigor index were transformed into arcsin 5% vigor index for statistical analysis, and in each cultivar means with the same letter are statistically identified by Tukey's test at 5% level.*

a consequence of ventilation results in an increase of vigor index and germination percentage of the heavier blocks. These results are according to Ferraz[5] and Cicero[4], found in rice seeds.

The utilization of the seed blower depends on the fuzz pre-cleaning according to the methodology recognized by Silva[10]. The fuzz pre-cleaning facilitates storage and transport of caryopses, reduces weight by 17% and volume by 64%. In ideal conditions, the caryopses ventilation can eliminate 56% of impurities by weight. The association of pre-cleaning and cleaning of caryopses by ventilation reduces the initial weight by approximately 63% and volume by 64% providing more conditions for economical storage and transportation.

The principal advantage of using sugarcane caryopses cleaned by ventilation is that this process increases germination and vigor. It also increases the efficiency on the use of soil and greenhouse area. Increased germination would result to better conditions in evaluating the seedlings of superior standard thus improving the breeding program.

CONCLUSIONS

According to the data obtained the following conclusions can be made: the ventilation of sugarcane caryopses eliminates the physical impurities; the ventilation increases the germination percentage and vigor of sugarcane caryopses; and the elimination of impurities decreases the concentration of undesired microorganism existing in the caryopses.

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EL EFECTOS DE LA VENTILACION DE CARIOPSES DE CAÑA DE AZUCAR SOBRE GERMINACION Y VIGOR.

J. Bleicher y H. Tokeshi

RESUMEN

Cariopses de 5 cultivares de caña de azucar fueron limpiados con un limpiador de semillas a efectos de eliminar impurezas fisicas, con una fuerza de ventilacion entre cero a 9,6 mg/cm^2. En el mejor tratamiento de limpieza, cerca de 56% de las impurezas fueron eliminadas, lo que redujo el peso inicial de los cariopses en 63%.

Los porcentajes de germinacion y el indice de vigor de los cariopses limpios en condiciones ideales fueron mayores que en el caso de los no tratados.

EFFECT ON VARIOUS AUXINS AND GIBBERELLIC ACID ON SUGARCANE CALLUS FORMATION AND THEIR SUBSEQUENT INFLUENCE ON DIFFERENTIATION AND ON CHROMOSOME NUMBER OF SUGARCANE PLANTLETS *IN VITRO*

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ABSTRACT

The effect of various auxins and gibberellic acid (GA_3) on callus formation and their subsequent influence on differentiation and on chromosome number of plantlet regenerated was studied. All auxins used were found to promote callus formation at certain concentration. Among these auxins, 2,4-D was found to be the most potent growth promoting substance that initiated callus formation.

The highest number of plantlets was regenerated from callus initially cultured at 5 mg/li 2,4-D, followed by 1 mg/l. The same pattern of response was observed with napthaline acetic acid (NAA). Increasing the amounts of 2,4-D and NAA up to 20 mg/l decreased the subsequent plantlet regeneration. Color variation among the plantlets was correlated to 2,4-D levels used.

Higher concentration of 2,4-D and NAA caused variation in chromosome number of regenerated plants.

INTRODUCTION

Tissue culture has numerous potential usage in sugarcane production and breeding. It allows rapid plant multiplication within a much shorter time from limited propagation material. Barba *et al*[1] reported that one sugarcane spindle may produce planting material to plant a hectare using mass propagation via tissue culture.

Since the start of sugarcane tissue culture by L.G. Nickell[2], variable plants had already been observed. Variants which were resistant to mosaic virus have been

obtained from susceptible variety to that disease via tissue culture by Coleman[3] in 1970. Lin and Chen[4] reported an increase of sugar content by 2-12% over the donor in plants derived from tissue culture. Colored variants were frequently observed among the regenerated plantlets. This means that plantlets derived from tissue culture are not homogeneous.

If plant tissue culture is intended to be used as a research tool in some physiological studies for crop improvement and as a means of rapid propagation, a certain degree of uniformity among regenerated plantlets must be attained. On the other hand, wider variability among plantlets obtained from tissue culture is important to increase the genetic base. The aim of this study is to find out the effect of some growth regulators on callus formation and its subsequent influence on differentiation and chromosome numbers and other variations among the derived plantlets.

MATERIALS AND METHODS

The top portion of sugarcane plant CAC 57-13 bearing the spindle tissues were used as the source of planting materials for the investigation. The first 3 or 4 mature outer leaves of the surfaced sterilized top were removed leaving the inner soft portion bearing about 6 whorls of leaves approximately 9 to 10 mm in diameter each. For uniformity of planting materials, the regular cut was about 3 mm (approximately 3-4 gram) excised crosswise from the middle of the spindle.

Initiation of callus from the cut tissues was achieved on a based medium of Murashige and Skoog[5] modified for sugarcane (Barba *et al*[1,6]). Growth regulators were incorporated according to the treatment.

The following treatments were tested:

Growth regulator	Concentration (mg/li)		
GA	1	5	20
NAA	1	5	20
IAA	1	5	20
2,4-D	1	5	20
Control	0	0	0

Each growth regulator was incorporated separately in the M.S. medium for callus formation at the levels mentioned above. Morphological differences in cultures among treatments were noted at weekly intervals with final observation at 4 weeks.

Callus from 4 week cultures were subcultured to the medium for differentiation. Two months after, plantlets were harvested for observation, chromosome counts of both the donor plant and the regenerated plantlets were made employing leaf squash technique for counting chromosomes (Price[7]).

RESULTS AND DISCUSSION

The effect of different growth regulators which were tried separately at different concentrations on the formation and growth of sugarcane callus are presented in Table 1.

TABLE 1. Response of sugarcane tissue to different growth regulators incorporated in modified MS medium and their effect on number of regenerated plants.

Growth regulators (mg/li)	One month cultures	No. of plants
GA		
1	Dead	0
5	Leaf tissue elongation	0
20	Leaf tissue elongation; greening	0
IAA		
1	No change	0
5	Slight callus and root formation	0
20	Further callus and root development	6
NAA		
1	Slight root and leaf points formation	9
5	Excessive roots and slight callus	11
20	Excessive roots and slight callus formation	7
2,4-D		
1	Callus and leaf points all over the cut ends	16
5	Excessive callus all over	26
20	Excessive soft and white callus all over	12
Control	Dead	0

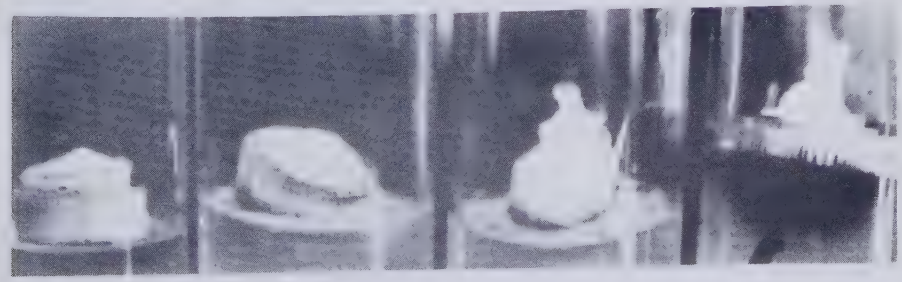

FIGURE 1. Effects of GA on callus formation of sugarcane tissue culture. Concentration from left: 0, 1, 5 and 20 mg/li.

GA did not promote observable callus formation in any concentration used. Instead, it promoted tissue maturation at levels 5 and 20 mg/li. There was only slight bulging at the cut ends of the leaf tissue which became distinct during the first week. Tissue elongation and greening however, were noted in all the concentrations used (See Fig. 1). At three levels of NAA tested, it was observed that root

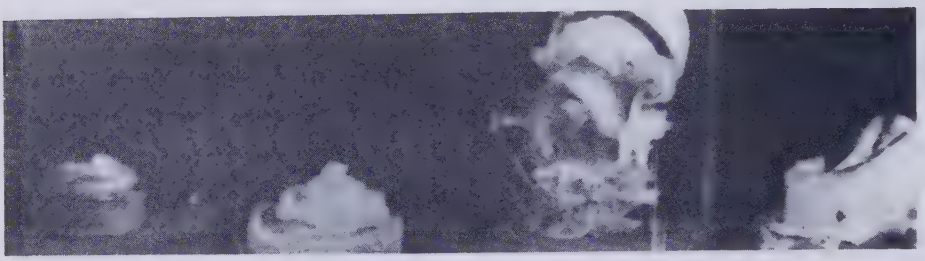

FIGURE 2. Effects of NAA on formation and growth of sugarcane callus culture. Concentration from left: 0, 1, 5 and 20 mg/li.

formation was favored (Fig. 2). While both 5 and 20 mg/l concentration showed excessive root formation, bigger but shorter roots were formed at 20 mg/l. There was only slight callus formation in some portion of the cut ends which was more prominent in culture at 5 and 20 mg/l. Leaf points and plantlets which seemed to originate from the leaf tissue were also noted. IAA slightly enhanced callus formation (Fig. 3). Root formation was also favored as with NAA, especially at

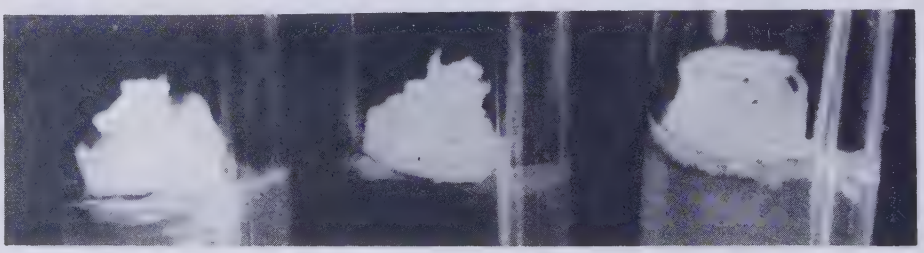

FIGURE 3. Effects of IAA on formation and growth of sugarcane callus culture. Concentration from left: 1, 5 and 20 mg/li.

levels 5 and 20 mg/l. There was slight callus formation on tissue that did not form roots at the same level. More likely, the early appearance of roots in the cultures suppressed the formation of more callus. 2,4-D at all concentration tested, promoted callus formation (Fig. 4). At 5 and 20 mg/l, almost the same excessive callus formation was observed. However some parts of the tissue, especially the outermost whorl was soft and slimy at 20 mg/l. Some portion was dehydrated and dried. This drying maybe due to the herbicidal effect of 2,4-D on the tissue. Early leaf points development all over the cut ends was observed aside from callus formation at 1 mg/l. The control showed no apparent change. Initial tissues turned brown and eventually died.

The results indicated that auxins, especially NAA, IAA, or 2,4-D are essential

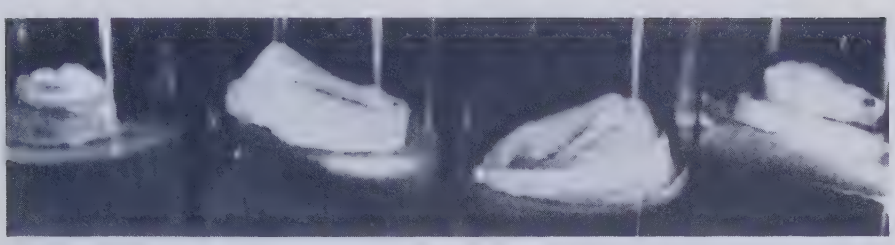

FIGURE 4. Effects of 2, 4-D on formation and growth of sugarcane callus culture. Concentration from left: 0, 1, 5 and 20 mg/li.

for callus initiation and proliferation of sugarcane tissues cultured *in vitro.* Among these auxins, 2,4-D showed the highest potency to initiate callus formation. This confirms the previous report by Nishi *et al*[8] that 2,4-D, a synthetic auxin is generally more effective for callus induction than NAA or IAA. However, NAA and IAA at 5 and 20 mg/li could substitute partly for 2,4-D in induction of callus in sugarcane.

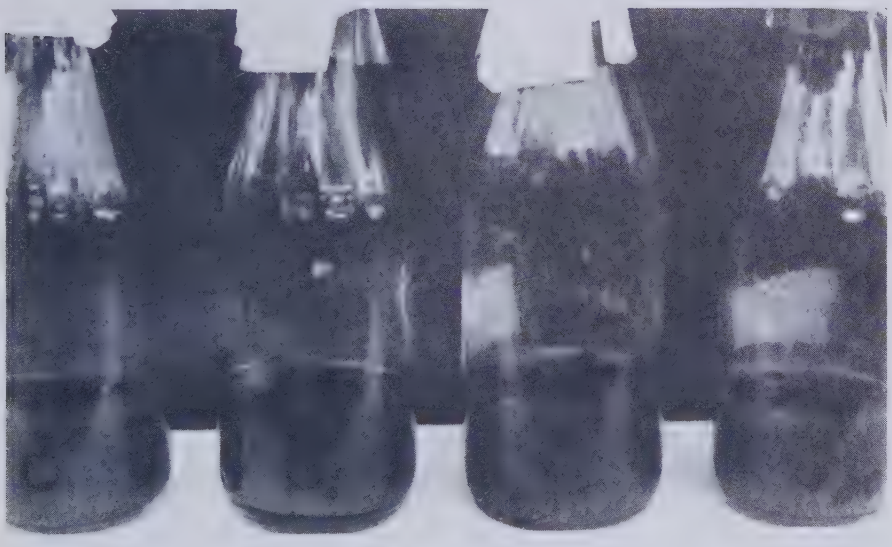

FIGURE 5. Effects of GA treatment during callus formation on sugarcane plantlet regeneration. Concentration from left: 0, 1, 5 and 20 mg/li.

It has been shown in the preceding experiment that GA did not promote observable callus formation. The result suggests that GA has no stimulative effect on cells of sugarcane preparatory to plantlet regeneration (Fig. 5). IAA effected minimal plantlet regeneration at 20 mg/l (Fig. 6). On the other hand, NAA and 2,4-D showed regeneration of plantlets at all concentrations used (Fig. 7 and 8). s,4-D at 5 mg/li produced the highest number of plantlets (26), followed by 2,4-D at 1 mg/l (16). The same pattern of response was observed on NAA with the highest number of plantlets obtained at 5 mg/li. A decrease in number of plantlets was

FIGURE 6. Effects of IAA treatment during callus formation on sugarcane plantlet regeneration. Concentration from left: 0, 1, 5 and 20 mg/li.

FIGURE 7. Effects of NAA treatment during callus formation on sugarcane plantlet regeneration. Concentration from left: 0, 1, 5 and 20 mg/li.

observed at 20 mg/li. Regenerated plantlets varied in color from normal green to albino (Fig. 9). There was also incidence of variegated or striped plantlets. Table 2 shows that the percentage of color variation was influenced by the concentration of 2,4-D incorporated in the medium for callus formation. While color variation had been observed previously, there was no definite 2,4-D level which had been correlated to color variants (Barba *et al*[1]). In this study the highest percentage of color

FIGURE 8. Effects of 2, 4-D treatment during callus formation on sugarcane plantlet regeneration. Concentration from left: 0, 1, 5 and 20 mg/li.

FIGURE 9. Normal green and albino plantlets regenerated from sugarcane callus cultures from 2, 4-D treatment.

variants was observed at 20 mg/l, 2,4 D. At lower concentrations (1 and 5 mg/l) the percentage of color variants was also lower. There was no color variants observed in the plantlets that proliferated in the medium with IAA and NAA as growth regulators.

TABLE 2. Percentage of color variants on plantlets regenerated from callus initially cultured on media with various auxin additive at different levels

Auxin source (mg/li)	Normal Green	Color Variants (%) Albino	Variegated
IAA			
1	0	0	0
5	0	0	0
20	100	0	0
NAA			
1	100	0	0
5	100	0	0
20	100	0	0
2,4-D			
1	94.4	5.6	0
5	92.2	7.8	0
20	77.3	18.7	4

Effect on chromosome number

In the chromosome level, there were slight variations on ploidy plantlets derived from different treatments. Plantlets from treatments with 2,4-D chromosome counts (2n) varied from 91-120, with the highest chromosome frequency counts of the ranges 106-110 and 96-100 at 1 and 20 mg/l, 4-D, respectively. Those treated with NAA have highest chromosome frequency counts within the ranges of 106-110 and 116-120 at 1 and 20 mg/li NAA, respectively. The chromosome counts in plantlets from both treatments varied from the donor plant with highest chro-

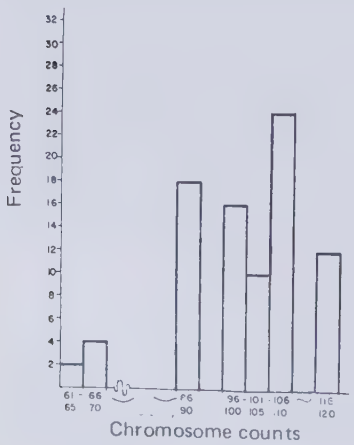

FIGURE 10. Chromosome counts

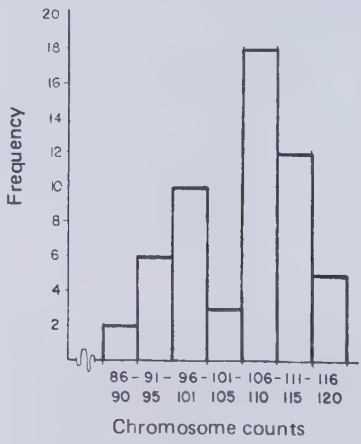

FIGURE 11. Chromosome counts

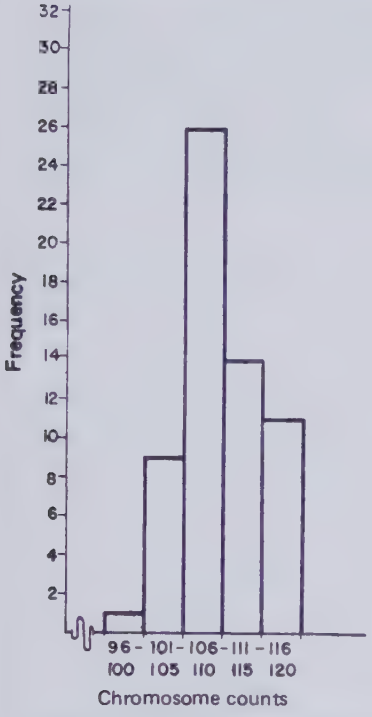

FIGURE 12. Chromosome counts

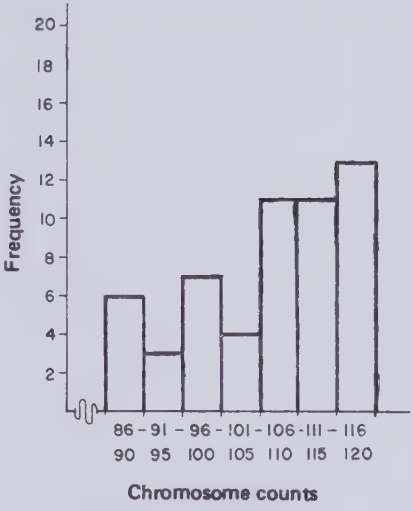

FIGURE 13. Chromosome counts

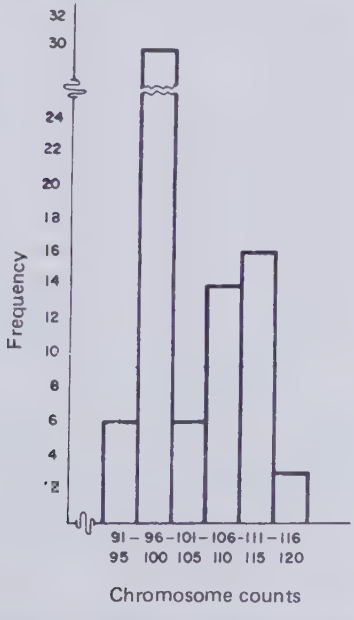

FIGURE 14. Frequencies of chromosome counts from sugarcane plantlets derived from callus initially culture in medium with 2,4-D at 20 mg/l.

mosome counts ranging from 106-110 (Figure 10-13). The results suggested that 2,4-D and NAA at highest concentration (20 mg/li) could cause variation in ploidy of tissue culture of sugarcane plants. Variation in ploidy among plantlets within treatments was observed (Table 3). Variation in ploidy at lower level implied that it is not growth regulator tested that cause variation. It may have been caused by some other factors. Heinz and Mee[9] have shown an appreciable chromosome variation among subclones derived from sugarcane tissue cultures.

TABLE 3. Chromosome number of several plants derived from callus of sugarcane variety CAC 57-13 treated with 2,4-D and NAA both at 1 and 20 mg/li concentrations

Derived plantlets*	Number of chromosomes
DONOR PLANT	86-120
2,4-D at 1 mg/li	
–1	102-120
–2	102-120
–3	100-120
–4	100-118
–5	102-118
2,4-D at 20 mg/li	
–1	96-106
–2	96-108
–3	90-120
–4	96-120
–5	98-116
NAA at 1 mg/li	
–1	90-118
–2	96-114
–3	90-120
–4	96-120
NAA at 20 mg/li	
–1	96-116
–2	96-116
–3	96-116
–4	96-116

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EFECTO DE VARIAS AUXINAS Y ACIDO GIBERELICO EN LA FORMACION DE CALLOS Y SU POSTERIOR INFLUENCIA EN LA DIFERENCIACION Y EN LOS NUMEROS CROMOSOMICO DE LAS PLANTULAS OBTENIDAS IN VITRO.

C. Linga-Morales y R.C. Barba

RESUMEN

Se estudió el efecto de varias auxinas y del acido geberelico (AG_3) en la formacion de callos y su posterior influencia en la diferenciacion y los numeros cromosomicos de las plantulas regeneradas. Todas las auxinas utilizadas promovieron la formacion de callos a ciertas concentraciones. Entre estas auxinas, el 2, 4-D fue el mas potente promotor del crecimiento que inició la formacion de callos.

El mayor numero de plantulas fue regenerado a partir de callos cultivados inicialmente con 5mg/1 de 2,4-D, seguido por 1mg/1. El mismo tipo de respuesta se observó para el acido naftalenacetico (NAA). Si se incrementa la cantidad de 2,4-D y de NAA hasta 20mg/1, decrece la subsiguiente regeneracion de plantulas. El nivel de 2,4-D afectó ademas la coloracion de las plantulas.

Concentraciones mas elevadas de 2,4-D y NAA fueron causa de variacion en los numeros cromosomicos de las plantulas obtenidas.

DESIGN AND STATISTICAL ANALYSIS OF EXPERIMENTS ON THE SELECTION OF OPTIMUM CULTURE MEDIUM IN SUGARCANE TISSUE CULTURE TECHNIQUE

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ABSTRACT

The systematic investigation on the subject of sieving sugarcane culture media for the best, by means of biometry, has not yet been made. Even though a lot of works and achievements have been done on the refinement of sugarcane tissue culture technique, there still remain so many problems to be solved in the selection for the optimum sugarcane culture medium.

Confounding and fractional replication techniques were introduced in this paper because they save a lot of time, labor, and laboratory facilities. They have high efficiency for experimentation and give us more information from a limited scale of experiment. First, we introduced the confounding of a set of 2^{p-1} interactions of a 2^n factorial experiment with 2^p incomplete blocks of 2^{n-p} plots each. Then, we let all higher order interactions go to make up the estimate of error so we can have enough precision and information about all main effects and first order interactions from a non-replicated design of experiment. Secondly, we introduced the fractional replication design. It is a special kind of confounding design where interactions were not being confounded individually but by groups. Only a fraction of 2^n factorial treatment combinations was included in a fractional replication design, while main effect, first order interactions, and higher order interactions were pooled altogether in an effect alias subgroup. The mixed or confounded effect of that subgroup stand for the main effect or the first order interaction.

Theoretical or practical examples were given in this study. Through these examples, it was shown that sucrose and 2,4-D are vital as chemical components of a sugarcane culture medium while kinetin was not as

it even inhibits the growth of callus tissues. Sucrose and 2,4-D interactions were significantly positive, while sucrose and kinetin, as well as kinetin and 2,4-D, were significantly negative.

INTRODUCTION

It is well known that the use of sugarcane tissue culture technique is important. The refinement of the technique requires biometry as shown by Cornish[2], Finney[3,4], Fisher[3,6], Kompthorne[7], Kishen[18], Wang-Tsien-ming[10,11,12] and Yates[13] Nowadays however, neither the biometricians nor the biologists pay much attention to the statistical method on the systematic selection of optimum culture medium for this technique. This brings a need for biometricians and biologists to work together seriously in order to make it a great success.

DISCUSSION

For the systematic selection of optimum culture medium of sugarcane tissue culture, the confounding technique of factorial experiments must be used. In general, there are eight main chemical components of a sugarcane tissue culture medium. They are potassium nitrate, ammonium nitrate, potassium dihydrogen phosphate; $Na_2EDTA + FeSO_4$ vitamin B_1, 2,4-D, kinetin and sucrose. They corresponded to eight factors indicated as A,B,C,D,E,F,G and H respectively. A,B,C . . . each stands for the main effect of potassium nitrate, ammonium nitrate, potassium dihydrogen phosphate . . . respectively. AB, BC . . . each stands for the first order interaction of potassium nitrate, ammonium nitrate and potassium dihydrogen phosphate . . . respectively. ABC . . . . . stands for the second order interaction of potassium nitrate, ammonium nitrate and potassium dihydrogen phosphate and so forth. Let us take a design of 2^8 factorial experiment as an example. There are eight factors of two levels each in this experiment. As a whole, there are 256 treatment combinations (i.e 256 kinds of culture media) replication. All high order interaction make up the estimate of exprimental error. Sometimes it is not so easy to have uniformity of experimental conditions and experimental materials. In such case, we have to divide our whole block into 4 incomplete blocks of 64 treatment combinations each, or into 8 incomplete blocks of 32 treatment combinations each. Accordingly, we have to sacrifice three degrees of freedom of interactions in the case of 8 incomplete blocks. Those interactions to be sacrificed are not selected arbitrarily but with careful considerations. The higher the order of interaction, the less important it is. If we want to sacrifice a set of 3 or 7 interactions, we can not sacrifice a set of the highest interactions. What we have to do is to select 1 or 3 somewhat high order interactions and choose another similar order interaction to interact with them to get 1 or 3 generalized interactions. Thus, a set of 3 or 7 interactions to be sacrificed will be formed by adding the generalized interactions to the original interactions and the interacting interaction. With a set of 3 interactions, there are only two interactions with free choice. Once we have chosen two definite interactions, the third one in the same set is fixed. Similarly, once we have chosen six definite interactions, the seventh one in the same set of 7 interactions is fixed.

According to the definite set of interactions to be sacrificed, we may work out all the treatment combinations for each incomplete block. An illustration of a 2^8 factorial experiment where the tissue media are composed of eight factors of two levels each chemical components is shown in Table 1.

TABLE 1. Eight factors of two levels each of a 2^8 sugarcane tissue culture factorial experiment.

Sign of factor	Significance of factor	Significance of level	Sign of level
A	Potassium nitrate	nil 1900 mg/liter	a_0 a_1
B	Ammonium nitrate	nil 1650 mg/liter	b_0 b_1
C	Potassium dihydrogen phosphate	nil 170 mg/liter	c_0 c_1
D	$Na_2EDTA + FeSO_4$	nil 37.3 + 27.8 mg/liter	d_0 d_1
E	Vitamin B_1	nil 0.4 mg/liter	e_0 e_1
F	2, 4-D	nil 3 mg/liter	f_0 f_1
G	Kinetin	nil 1 mg/liter	g_0 g_1
H	Sucrose	nil (or 10 g/l) 20 g/liter	h_0 h_1

There are eight factors of two levels to make up 256 treatment combinations, i.e. formula of sugarcane tissue culture media. For example, the formula of $a_1b_0c_1d_1e_0f_0g_1h_1$ or acdgh stands for the adding of 1900 mg of potassium nitrate, 170 mg of potassium dihydrogen phosphate, 37.3 mg of Na_2EDTA + 27.8 mg. of $FeSO_4$ 1 mg of kinetin and 25 g of sucrose to 1000 ml. of distilled water to make such a medium. The formula for (1) or $a_0b_0c_0d_0e_0f_0g_0h_0$ stands for that treatment combination with lower levels for all factors.

In order to get more uniformity of experimental conditions and experimental materials, we design to divide our whole block of 256 treatment combinations into 4 incomplete blocks of 64 combinations each. The main effects and first order interactions should not be sacrificed or confounded. Hence we try ADEF and CDFH, two third order interactions, as they are considered to be less important. If we want to know which treatment combinations enter each of the four incomplete blocks, we must choose any two of the set of three confounded interactions. As mentioned above, if ADEF and CDFH are confounded, ACEH is also confound-

ed at the same time. ACEH is the generalized interaction, when ADEF and CDFH are mutually interacted. Suppose we choose ADEF and CDFH as "defining interactions". Any treatment combination, among the 256 having even numbers common to both "defining interactions" enters the '++' or the first incomplete block. Any treatment combination having even number common to ADEF and odd number common to CDFH, enters the '+ -' or the second incomplete block. Any treatment combination having odd number common to ADEF, but even number common to CDFH, enters the '−+' or the third incomplete block. Any treatment combination having odd numbers common to both "defining interactions" enters the '−−' or the fourth incomplete block. To each of the 4 incomplete blocks there are 64 treatment combinations as shown in Table 2.

TABLE 2. 2^8 factorial design with interactions ADEF, CDFH and ACEH confounded to give 4 incomplete blocks of 64 treatment combinations each.

'++' or the first incomplete block	'+−' or the second incomplete block	'−+' or the third incomplete block	'−−' or the fourth incomplete block
(1)	c	a	ac
b	bc	ab	abc
acd	ad	cd	d
abcd	abd	bcd	bd
ae	ace	e	ce
abe	abce	be	bce
cde	de	acde	ade
bcde	bde	abcde	abde
acf	af	cf	f
abcf	abf	bcf	bf
df	cdf	adf	acdf
bdf	bcdf	abdf	abcdf

TABLE 2 contd.

'++' or the first incomplete block	'+−' or the second incomplete block	'−+' or the third incomplete block	'−−' or the fourth incomplete block
cef	ef	acef	aef
bcef	bef	abcef	abef
adef	acdef	def	cdef
abdef	abcdef	bdef	bcdef
g	cg	ag	acg
bg	bcg	abg	abcg
acdg	adg	cdg	dg
abcdg	abdg	bcdg	bdg
aeg	aceg	eg	ceg
abeg	abceg	bcg	bceg
cdeg	deg	acdeg	adeg
bcdeg	bdeg	abcdeg	abdeg
acfg	afg	cfg	fg
abcfg	abfg	bcfg	bfg
dfg	cdfg	adfg	acdfg
bdfg	bcdfg	abdfg	abcdfg
cefg	efg	acefg	aefg
bcefg	befg	abcefg	abefg
adefg	acdefg	defg	cdefg
abdefg	abcdefg	bdefg	bcdefg
ch	h	ach	ah
bch	bh	abch	abh
adh	acdh	dh	cdh
abdh	abcdh	bdh	bcdh
aceh	aeh	ceh	eh
abceh	abeh	bceh	beh
deh	cdeh	adeh	acdeh
bdeh	bcdeh	abdeh	abcdeh
afh	acfh	fh	cfh
abfh	abcfh	bfh	bcfh
cdfh	dfh	acdfh	adfh
bcdfh	bdfh	abcdfh	abdfh

TABLE 2. contd.

'++' or the first incomplete block	'+−' or the second incomplete block	'−+' or the third incomplete block	'−−' or the fourth incomplete block
efh	cefh	aefh	acefh
befh	bcefh	abefh	abcefh
acdefh	adefh	cdefh	defh
abcdefh	abdefh	bcdefh	bdefh
cgh	gh	acgh	agh
bcgh	bgh	abcgh	abgh
adgh	acdgh	dgh	cdgh
abdgh	abcdgh	bdgh	bcdgh
acegh	aegh	cegh	egh
abcegh	abegh	bcegh	begh
degh	cdegh	adegh	acdegh
bdegh	bcdegh	abdegh	abcdegh
afgh	acfgh	fgh	cfgh
abfgh	abcfgh	bfgh	bcfgh
cdfgh	dfgh	acdfgh	adfgh
bcdfgh	bdfgh	abcdfgh	abdfgh
efgh	cefgh	aefgh	acefh
befgh	bcefgh	abefgh	abcefgh
acdefgh	adefgh	cdefgh	defgh
abcdefgh	abdefgh	bcdefgh	bdefgh

The key for the statistical analysis of the above design is given in Table 3.

The sum of squares for main effect A is calculated by subtracting those 128 treatment combinations with potassium nitrate from those 128 treatment combinations with potassium nitrate from those 128 treatment combinations without potassium nitrate and by squaring the diffrence and then dividing it by 256. The sums of squares for the other 7 main effects are calculated in the same way. The sum of squares for interaction AB is calculated by subtracting those 128 treatment combinations with or without both potassium nitrate and ammonium nitrate from

TABLE 3. Analysis of variance for the 2^8 factorial design with ADEF, CDFD & ACEH compounded

Cause of variation	Degree of freedom
incomplete blocks	3
main effects	8
first order interactions	28
error	216
Total	255

those 128 treatment combinations with either potassium nitrate or ammonium nitrate, by squaring that difference and then dividing by 256. The sums of squares for the other 27 first order interactions are calculated in the same way. Those main effects tested to be significant are said to be important while those tested to be insignificant are said to be unimportant. Those first order interactions tested to be significant are said to have interaction between two related factors. Those first order interactions tested to be insignificant are said to be of no interaction between two related factors. If it happens to have a significant positive first order interaction, it means that the effect of the presence of both two factors is better than that of the presence of a single factor.

If we still think the size of the above incomplete blocks too large to give us a high uniformity of experimental conditions and experimental materials, we can further divide our whole block into 8 incomplete blocks of 32 plots each with seven interactions confounded. Let us consider ADEF, CDFH and ACEH as a basis and add one more interaction, say BEG to interact with them to produce generalized interactions, i.e. ABDFG, BCDEFGH, and ABCGH. They are all together seven interaction to be confounded. No main effects nor first order interactions are sacrificed, so it is rather a good design. Choose BEG, ADEF and CDFH as "defining interactions", we can have all 32 treatment combinations entering the corresponding 8 incomplete blocks. Any treatment combination having odd number common to BEG, even number common to both ADEF and CDFH enters the '+++' or the first incomplete block. Any treatment combination having odd number common to both BEG and CDFH, given number common to ADEF enters the '++−' or the second incomplete block. Similarly, we can work out the remaining 6 incomplete blocks. Table 4 shows the details of this design.

It is necessary to compare the advantages and disadvantages between two designs where a set of 3 or 7 interactions is confounded. The former sacrifices less information of interactions but the latter has a better estimation of experimental error, and a better control of the uniformity of experimental conditions and experimental materials. If it is a field experiment where the size of incomplete blocks is closely related to the magnitude of experimental error, the latter design is superior to the former. If it is a tissue culture medium experiment where the size of incomplete blocks is not so closely related to the magnitude of experimental error, the latter design is superior to the former. If it is a tissue culture medium experiment where the size of incomplete blocks is not so closely related to the magnitude of experimental error, the choice of either the latter or the former design will depend upon the degree of variation of experimental conditions and experimental materials.

If we think the number of treatment combinations of a 2^8 factorial experiment is too large to handle, one may use a fraction of a complete replicate. The particular fraction of treatment combinations of a factorial experiment to use has been discussed by Finney[3,4] and Kompthorne[7] under the topic of fractional replication.

Let us suppose a 2^n factorial experiment with n factors of two levels each. If n is too large to handle, we choose a smaller number $p < n$. Then, instead of 2^n treatment combinations for the whole experiment we have only 2^p treatment combinations, only a fraction $2^{(n-p)}$ of the complete replicate. In this way we have saved 2^{n-p} treatment combinations.

When we use 1/2 replication, n is equal to $^{p+1}$. In order to let 2^{p-1} degrees of freedom to denote 2^{n-1} or 2^{p+1} effects, we must group the effects pair by pair so as to form "effect alias subgroup". Every two of effects within each effect

TABLE 4. 2^8 factorial design with interactions BEG, ADEF, CDFH, ACEH, ABDFG, BCDLFGH and ABCGH confounded to give 8 incomplete blocks of 32 treatment combinations.

'+++' or the first incomplete block	'++−' or the second incomplete block	'+−+' or the third incomplete block	'−++' or the fourth incomplete block	'+−−' or the fifth incomplete block	'−+−' or the sixth incomplete block	'−−+' or the seventh incomplete block	'−−−' or the eight incomplete block
b	bc	ab	(1)	abc	c	a	ac
abcd	abd	bcd	acd	bd	ad	cd	d
ae	ace	e	abe	ce	abce	be	bce
cde	de	acde	bcde	ade	bde	abcde	abde
abcf	abf	bcf	acf	bf	af	cf	f
bdf	bcdf	abdf	df	abcdf	cdf	adf	acdf
cef	ef	acef	bcef	aef	bef	abcef	abef
adef	acdef	def	abde	cdef	abcdef	bdef	bcdef
g	cg	ag	bg	acg	bcg	abg	abcg
acdg	adg	cdg	abcdg	dg	abdg	bcdg	bdg
abeg	abceg	beg	aeg	bceg	aceg	eg	ceg
bcdeg	bdeg	abcdeg	cdeg	abdeg	deg	acdeg	adeg
acfg	afg	cfg	abdfg	fg	abfg	bcfg	bfg
dfg	cdfg	adfg	bdfg	acdfg	bcdfg	abdfg	abcdfg
bcefg	befg	abcefg	cefg	abefg	efg	acefg	aefg
abdefg	abcdefg	bdefg	adefg	bcdefg	acdefg	defg	cdefg
bch	bh	abch	ch	abh	h	ach	ah
abdh	abcdh	bdh	adh	bcdh	acdh	dh	cdh
aceh	aeh	ceh	abceh	eh	abeh	bceh	beh
deh	cdeh	adeh	bdeh	acdeh	bcdeh	abdeh	abcdeh
abfh	abcfh	bfh	afh	bcfh	acfh	fh	cfh
bcdfh	bdfh	abcdfh	cdfh	abdfh	dfh	acdfh	adfh
efh	cefh	aefh	befh	acefh	bcefh	abefh	abcefh
acdefh	adefh	cdefh	abcdefh	defh	abdefh	bcdefh	bdefh
cgh	gh	acgh	bcgh	agh	bgh	abcgh	abgh
adgh	acdgh	dgh	abdgh	cdgh	abcdgh	bdgh	bcdgh
abcegh	abegh	bcegh	acegh	begh	aegh	cegh	egh
bdegh	bcdegh	abdegh	degh	abcdegh	cdegh	adegh	acdegh
afgh	acfgh	fgh	abfgh	cfgh	abcfgh	bfgh	bcfgh
cdfgh	dfgh	acdfgh	bcdfgh	adfgh	bdfgh	abcdfgh	abdfgh
befgh	bcefgh	abefgh	efgh	abcefgh	cefgh	aefgh	acefgh
abcdefgh	abdefgh	bcdefgh	acdefgh	bdefgh	cdefgh	cdefgh	defgh

The key for statistical analysis of the above design is given in Table 5.

TABLE 5. Analysis of variance for a 2^8 factorial design with BEG, ADEF, CDFH, ACEH, ABDFG, BCDFG, and ABCGH confounded.

Cause of variation	degree of freedom
Incomplete blocks	7
main effects	8
first order interactions	28
error	212
T o t a l	255

alias subgroup are not independent and are said to be confounded in the subgroup. In like manner, effect alias subgroups are composed of four, eight, ... and so on effects in 1/4, 1/8 replication designs. It should be followed carefully that no fractional replicational design will be used unless n is greater than 5 in a 2^n desgin.

If n is less than 5, the main effect is likely to be confounded with lower-order interaction. The fractional replication design is quite useful to deal with a 2^8 factorial design. Let us take for example a 2^8 factorial design with 1/4 replication. There are four effects to make up an effect alias subgroup. Suppose we select ABDEFH, ACDFG and BCEGH as three defining interactions to be confounded. Those three defining interactions together with the treatment total effect,make up. an initial effect alias subgroup:

$$1 = ABDEFH = ACDFG = BCEGH$$

1 is the treatment total effect and is said to be the "identity" of the subgroup. Once we have our initial effect alias subgroup, we can derive all the effect alias subgroups by taking any one effect to interact with the four member effects of the subgroup one by one. For example:

A = ABDEFH = CDFG = ABCEGH
. . . . . .
. . . . . .
AB = DEFH = BCDFG = ACEDGH
. . . . . .
. . . . . .
ABC = CDEFH = BDFG = AEGH
. . . . . .
. . . . . .
ABCD = CEFH = BFG = ADEGH
. . . . . .
. . . . . .
etc.

All four effects in a subgroup are mixed up and cannot be separated. The mixed-up effect is considered as the main effect or the lowest order interaction in that subgroup.

In order to work out all treatment combinations for the 1/4 fractional replication design, we must find out the principal block of 64 plots first. Any treatment combinations with even number common to the three defining interactions enter the principal block (see Table 6).

TABLE 6. Principal block of 64 plots in a ¼ fractional replication design of a 2^8 factorial experiment with ABDEFH, ACDFG and BCEGH as three defining interactions.

(1)	af	abg	bfg	bh	abfh	agh	fgh
abc	bcf	cg	acfg	ach	efh	bcgh	abcfgh
ad	df	bdg	abdfg	abdh	bdfh	dgh	adfgh
bcd	abcdf	acdg	cdfg	cdh	acdfh	abcdgh	bcdfgh
be	abef	aeg	efg	eh	aefh	abegh	befgh
ace	cef	bceg	abcefg	abceh	bcefh	cegh	acefgh
abde	bdef	deg	adefg	adeh	defh	bdegh	abdefgh
cde	acdef	abcdeg	bcdefg	bcdeh	abcdefh	acdegh	cdefgh

If we do not like to take the set of 64 plots in the principal block, we can choose any treatment combination which is concluded in the 2^8 factorial scheme but not in the principal block, to "interact" each treatment combination and all generalized treatment combination. Then make up another 1/4 fractional replication design. Let us take ab as an example. The alterative 1/4 fractional replication design is given below:

ab	bf	g	afg	ah	fh	bgh	abfgh
a	acf	abcg	bcfg	bch	abcfh	acgh	cfgh
bd	abdf	adg	dfg	dh	adfh	abdgh	bdfgh
acd	cdf	bcdg	abcdfg	abcdh	bcdfh	cdgh	acdfgh
ae	ef	beg	abefg	abeh	befh	egh	aefgh
bce	cef	aceg	cefg	ceh	acefh	abcegh	bcefgh
de	adef	abdeg	bdefg	bdeh	abdefh	adegh	defgh
abcde	bcdef	cdeg	acdefg	acdeh	cdefh	bcdegh	abcdefgh

If we think the size of a 64-plot principal block still too large to handle, we go a little further to confound three more effect alias subgroups, i.e.:

$$EFG = ABDGH = ACDE = BCFH$$
$$ABEG = DFGH = BCDEF = ACH$$
$$ABF = DEH = BCDG = ACEFGH$$

TABLE 7. Incomplete blocks for the ¼ fractional replication of the 2^8 factorial design.

I (principal block)	II (ad X I)	III (be X I)	IV (abde X I)
(1)	ad	be	abde
abc	bcd	ace	cde
bdef	abef	df	af
acdef	cef	abcdf	bcf
deg	aeg	bdg	abg
abcdeg	bceg	acdg	cg
bfg	abdfg	efg	adefg
acfg	cdfg	abcefg	bcdefg
abdh	bh	adeh	eh
cdh	ach	bcdeh	abceh
aefh	defh	abfh	bdfh
bcefh	abcdefh	cfh	acdfh
abegh	bdegh	agh	dgh
cegh	acdegh	bcgh	abcdgh
adfgh	fgh	abdefgh	befgh
bcdfgh	abcfgh	cdefgh	acefgh

Any treatment combination appearing in the principal block in Table 6 having even number common to the three confounded effect alias subgroups enters the principal block in Table 7. Any treatment combination not in principal block I, say "ad" interacting every one treatment combination to form incomplete block II, any treatment combination not in incomplete block I and II, say "be" to form incomplete block III; any treatment combination not in incomplete block I, II, and III, say "abde" to form incomplete block IV. Table 7 shows the 4 incomplete blocks of 16 plots each for the 1/4 fractional replication of a 2^8 factorial design.

Table 8 shows the key for the statistical analysis of a fractional replication with 3 effect alias subgroups confounded for further division into 4 incomplete blocks of 16 plots.

This example is taken from the data of an experiment with a design shown in Table 1 and Table 7 and conducted by our collaborators Mai, et al. Table 9 shows the treatment combinations (or culture media) of 4 incomplete blocks and their callus tissue growth (mg) for five test tubes each.

The detailed analysis of variance is not given here but it is summarized in Table 10.

The growth of the callus tissue and its increment are attributed to sucrose and 2,4-D. Kinetin gave inhibitory effects. The interactions of 2,4-D and Kinetin, and of sucrose and kinetin are significantly negative while that of 2,4-D and sucrose is significantly positive. Other main effects and first order interactions do not reach the level of significance. It may be due to the fact that the lower level of sucrose factor due to no application of sucrose, has resulted in deficiency of carbon nut-

TABLE 8. Analysis of variance for a ¼ fractional replication with 3 effect alias subgroups confounded.

Cause of Variation	Degree of freedom	Effect alias subgroups
Incomplete blocks	3	EFG = ABDGH = ACDE = BCFH ABEG = DFGH = BCDEF = ACH ABF = DEH = BCDG = ACEFGH
Main effects	8	A = BDEFH = CDFG = ABCEGH B = ADEFH = ABCDFG = CEGH C = ABCDEFH = ADEG = BEGH D = ABEFH = ACFG = BCDEGH E = ABDFH = ACDEFG = BCGH F = ABDEH = ACDG = BCEFGH G = ABDEFGH = ACDF = BCEH H = ABDEF = ACDFGH = BCEG
First order interactions	28	AB = DEFH = BCDFG = ACEGH AC = BCDEFH = DFG = ABEGH AD = BEFH = CFG = ABCDEGH . . . . FH = ABDE = ACDGH = BCEFG GH = ABDEFG = ACDFH = BCE
Error	24	ABCD = CEFH = BFG = ADEGH . . . . CFGH = ABCDG = ADH = BEF
Total	63	

rients and lack of enough osmotic pressure to bring forth the growth of the callus tissue. Lack of enough osmotic pressure causes the death of the inoculated spindle tissue. Deficiency of carbon nutrients (or sucrose) causes no growth at all or nor further growth of the callus tissue.

Let us consider a theoretical example of sugarcane tissue culture medium selection experiment of 5 comparatively important factors of 3 levels each. We choose at random, the 1st, 3rd, 6th, 9th, 12th columns of a L_{27} (3^{13}) orthogonal design to represent the corresponding levels of 5 factors, i.e. potassium nitrate, po-

TABLE 9. Table showing the Treatment combinations and their callus tissue growth(mg), for a period of 21 days after innoculation of sugarcane spindle pieces, of 4 incomplete blocks of the 4^{-1} fractional replication of a 2^8 fractorial design.

I		II		III		IV	
(1)	mg.	ad	mg. 0	be	mg. 0	abde	mg. 0
abc	0	bcd	0	ace	0	cde	0
bdef	0	abef	0	df	0	af	0
acdef	0	cef	0	abcdf	0	bcf	0
deg	0	aeg	0	bdg	0	abg	0
abcdeg	0	bceg	0	acdg	0	cg	0
bfg	0	abdfg	0	efg	0	adefg	0
acfg	0	cdfg	0	abcefg	0	bcdefg	0
abdh	69.1	bh	0	adeh	0	eh	16.2
cdh	17.1	ach	19.1	bcdeh	0	abceh	15.2
aefh	325.5	defh	513.2	abfh	443.6	bdfh	673.2
bcefh	421.2	abcdefh	889.0	cfh	142.2	acdfh	1486.3
abegh	52.2	bdegh	2.5	agh	17.3	dgh	0
cegh	8.4	acdegh	156.5	bcgh	0	abcdgh	219.7
adfgh	170.3	fgh	168.8	abdefgh	108.2	befgh	157.5
bcdfgh	152.7	abcfgh	236.0	cdefgh	70.8	acefgh	133.3
Total	1216.5	Total	1985.1	Total	782.1	Total	2701.2

TABLE 10. Analysis of variance of the 4^{-1} fractional replication of a 2^8 factorial experiment shown in Table 9.

Cause of Variation	Degree of Freedom	Sum of Squares	Variances	F– Value	Level of Significance
Incomplete Blocks	3	134792.76	44930.92		
A	1	62331.37	62331.37	1.84	–
B	1	593.53	593.53	–	–
C	1	23451.57	23451.57	–	–
D	1	87934.49	87934.49	2.60	–
E	1	13980.11	13980.11	–	–
F	1	472432.84	472432.84	13.92	**
G	1	178136.76	178136.76	5.2	*
H	1	698248.25	698248.25	20.6	**
AB	1	8693.23	8693.23	–	–
AC	1	1268.25	1268.25	–	–
AD	1	15665.65	15665.65	–	–
AE	1	16176.66	16176.66	–	–
AF	1	15249.17	15249.17	–	–
AG	1	13563.52	13563.52	–	–
AH	1	62331.37	62331.37	1.84	–
BC	1	2439.13	2439.13	–	–
BD	1	9862.98	9862.98	–	–
BE	1	6571.13	6571.13	–	–
BF	1	43.73	43.73	–	–
BG	1	701.59	701.59	–	–
BH	1	593.53	593.53	–	–
CD	1	41468.23	41468.23	1.2	–
CE	1	701.59	701.59	–	–
CF	1	7497.39	7497.39	–	–
CG	1	6571.38	6571.38	–	–
CH	1	25684.07	25684.07	–	–
DE	1	20682.04	20682.04	–	–
DF	1	9783.14	9783.14	–	–
DG	1	72758.32	72758.32	2.14	–
DH	1	62331.37	62331.37	1.84	–
EF	1	9094.01	9094.01	–	–
EG	1	2439.13	2439.13	–	–
EH	1	4008.48	4008.40	–	–
FG	1	252091.86	252091.86	7.4	*
FH	1	472432.84	472432.84	13.9	**
GH	1	178136.76	178136.76	5.2	*
Error	24	814799.75	33949.99		
Total	63	3795679.00			

* *denotes 5% significance*
** *denotes 1% significance*

tassium dihydrogen phosphate > Vitamin B_1, 2,4-D and sucrose. The details are tabulated in Table 11.

TABLE 11. Treatments of a sugarcane tissue culture medium selection experiment.

Factor	Level		
	low	medium	high
potassium nitrate	1300 mg/l.	1900 mg/l.	2500 mg/l.
potassium dihydrogen phosphate	140 mg/l.	170 mg/l.	200 mg/l.
Vitamin B_1	0.1 mg/l.	0.4 mg/l.	0.7 mg/l.
2, 4-D	1 mg/l.	3 mg/l.	5 mg/l.
SUCROSE	10 g/l.	20 g/l.	30 g/l.

There are two replications in this experiment. To each replication there are all together 27 treatment combinations. Table 12 shows the three levels of each factor and the theoretical data for the 2 replications, the last column of the table is the absolute difference or the absolute range of the two replications for each treatment combinations. r is the range total for 27 treatment combinations. r is the mean of range. m_1 m_2 and m_3 represent the means of low, medium and high level for each factor. R is the range of m_1, m_2 and m_3 for each factor. Lu and Lin[9] worked out a table of range coefficients for 5% and 1% significant levels. Entering number of replications (i.e. 2) for completely random experiment of $L_{27}(3^{13})$ orthogonal design, we can find out 5% and 1% significant range coefficient to be 0.73 and 0.94, respectively. Using these two significant range coefficient to multiply the mean range, we can get the 5% and 1% least significant difference for every two level means of each factor. The details are given in Table 12.

Let us make use of the 5% and 1% least significant difference between two levels of a factor to test the significance of any two levels of each of the factors as indicated in Table 13, Table 14, Table 15, Table 16 and Table 17.

Among the 3 levels of potassium nitrate there are two comparisons of two levels being significant, i.e. 2500 mg/l, is very significant better than 1300 mg/l, 1900 mg/l is significantly better than 1300 mg/l.

Among the 3 levels of potassium dihydrogen phosphate, there are two com-

TABLE 12. Design and analysis of sugarcane tissue culture medium selection experiment of L_{27} (3^{13}) orthogonal design with the 1st, 3rd, 6th, 9th and 12th columns selected.

Sign of culture medium	Levels of factors					Weight of callus tissue (mg)			
	Potassium nitrate	Potassium dihydrogen phosphate	Vitamin B_1	2,4–D	Sucrose	Repl. I	Repl. II	Total	Range
1	1300mg/l	140mg/l.	0.1mg/l.	1mg/l.	10g/l.	102	130	232	28
2	1300	140	0.4	3	20	212	189	401	23
3	1300	140	0.7	5	30	300	292	592	8
4	1300	170	0.1	5	20	217	130	347	87
5	1300	170	0.4	1	30	143	214	357	71
6	1300	170	0.7	3	10	161	142	303	19
7	1300	200	0.1	3	30	139	113	252	26
8	1300	200	0.4	5	10	121	216	337	95
9	1300	200	0.7	1	20	212	140	352	72
10	1900	170	0.4	1	30	214	131	345	83
11	1900	170	0.7	3	10	176	151	327	25
12	1900	170	0.1	5	20	208	184	392	24
13	1900	200	0.4	5	10	201	196	397	5
14	1900	200	0.7	1	20	140	216	456	26
15	1900	200	0.1	3	30	204	174	378	30
16	1900	140	0.4	3	20	201	196	397	5
17	1900	140	0.7	5	30	301	291	592	10
18	1900	140	0.1	1	10	174	181	355	7
19	2500	200	0.7	1	20	141	156	297	15
20	2500	200	0.1	3	30	203	184	387	19
21	2500	200	0.4	5	10	176	186	362	10
22	2500	140	0.7	5	30	299	304	603	5
23	2500	140	0.1	1	10	165	156	321	9
24	2500	140	0.4	3	20	231	251	482	20
25	2500	170	0.7	3	10	298	267	565	31
26	2500	170	0.1	5	20	274	196	470	78
27	2500	170	0.4	1	30	201	182	383	19

TABLE 13. The significant test results of pair comparison from 3 levels of potassium nitrate.

	level mean	level mean	
		m_2 202.2	m_3 215.0
m_1	176.3	25.9*	38.7**
m_2	202.2		12.8

TABLE 14. The significant test results of pair comparisons from 3 levels of potassium dihydrogen phosphate.

level mean		level mean	
		m_2 193.8	m_3 178.8
m_1	220.8	−27.0*	−42.0**
m_2	193.8		−15.0

TABLE 15. The significant test results of 3 levels of Vitamin B_1

level mean		level mean	
		m_2 192.3	m_3 227.1
m_1	174.1	18.2	53.0**
m_2	192.3		34.8**

TABLE 16. The significant test results of pair comparison among 3 levels of 2, 4-D

level mean		level mean	
		m_2 194.0	m_3 227.3
m_1	172.1	21.9	55.2**
m_2	194.0		33.3**

TABLE 17. The significant test results of pair comparisons among 3 levels of sucrose.

level mean		level mean	
		m_2 199.7	m_3 216.1
m_1	177.7	22.0	38.4**
m_2	199.7		16.4

TABLE 18. Table of range coefficients for 5% and 1% significant levels in different designs.

Number of Replications or Blocks	level of significance	Designs						
		$L_8(2^7)$	$L_{12}(2^{11})$	$L_{16}(2^{15})$	$L_{24}(2^{23})$	$L_{32}(2^{31})$	$L_{18}(3^7)$	$L_{27}(3^{13})$
Complete randomization (No. of replicates) 2	5 %	1.01	0.78	0.66	0.53	0.45	0.92	0.73
	1 %	1.49	1.10	0.91	0.72	0.61	1.21	0.94
3	5 %	0.51	0.40	0.35	0.28	0.24	0.48	0.39
	1 %	0.70	0.55	0.47	0.37	0.32	0.61	0.49
4	5 %	0.35	0.28	0.24	0.20	0.17	0.34	0.27
	1 %	0.48	0.38	0.33	0.26	0.22	0.43	0.34
5	5 %	0.28	0.22	0.19	0.14	0.13	0.27	0.22
	1 %	0.37	0.30	0.25	0.21	0.18	0.33	0.27
Randomized blocks (No. of blocks) 2	5 %	1.10	0.83	0.69	0.54	0.46	0.96	0.75
	1 %	1.66	1.18	0.96	0.73	0.62	1.26	0.97
3	5 %	0.55	0.43	0.36	0.28	0.24	0.50	0.37
	1 %	0.76	0.58	0.49	0.38	0.32	0.63	0.50
4	5 %	0.38	0.30	0.26	0.20	0.17	0.53	0.28
	1 %	0.52	0.40	0.35	0.27	0.23	0.44	0.35
5	5 %	0.30	0.23	0.20	0.16	0.13	0.27	0.22
	1 %	0.40	0.31	0.26	0.21	0.18	0.34	0.27

parisons of two levels being significant, i.e. 140 mg/l, is very significantly better than 200 mg/l, 140 mg/l is significantly better than 170 mg/l.

As a result, 0.7 mg/l of Vitamin B_1 is very significantly better than 0.4 mg/l and 0.1 mg/l, while there is no significant difference between 0.4 mg/l, and 0.1 mg/l.

As a result, 5 mg/l. of 2,4-D is very significantly better than either 3 mg/l. or 1 mg/l., while there is no significant difference between 3 mg/l. and 1 mg/l.

As a result, 30 g/l. of sucrose is very significantly better than 10 g/l.

Table 18 shows Lu and Lin's table of range coefficients for 5% and 1% significant levels in different designs.

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DISEÑO Y ANALISIS ESTADISTICO DE EXPERIMENTOS PARA SELECCIONAR MEDIOS OPTIMOS PARA LA TECNICA DE CULTIVO DE TEJIDOS EN CAÑA DE AZUCAR.

Wang Tsien-ming

RESUMEN

No se ha realizado hasta ahora una investigacion sistemática por métodos biométricos para mejorar los medios de cultivo. A pesar de que se han realizado numerosos trabajos y avances en el refinamiento de la técnica de cultivo de tejidos de caña de azúcar, todavia quedan muchos problemas por resolver en la selección de medios de cultivo óptimos.

En esta comunicacion se introducen las tecnicas de confundido y réplica fraccionada, puesto que ellas sirven el proposito de ahorrar tiempo, trabajo y facilidades en el laboratorio. Estas tecnicas tienen alta eficiencia para la experimentacion y dan mayor información para una escala experimental limitada. En primer lugar se presenta el confundido de 2^{p-1} interacciones de un factorial 2^n con 2^p bloques incompletos de 2^{n-p} parcelas cada uno. Posteriormente las interacciones de orden mas elevado se utilizan para estimar el error de manera de tener suficiente precision e información sobre los efectos principales y las interacciones de primer orden de un diseño experimental no replicado. A continuacion se presentan los diseños con replicación fraccionada, lo que es una clase especial de los diseños confundidos. En estos experimentos las interacciones no se confunden individualmente sino grupo por grupo. Solamente una fraccion, pero no todas las combinaciones de un factorial 2^n se incluyen en un diseño de replicación fraccionada, mientras que los efectos principales, las interacciones de primer orden y las interacciones de orden mayor se encuentran todas en un efecto de subgrupo; el efecto mixto o confundido de ese subgrupo permanece por el efecto principal o por la interacción de primer orden.

Se dan en esta comunicación ejemplos teóricos o de experimentos reales a los efectos de ilustrar la técnica. A traves de estos ejemplos surgiría que la sacarosa y el 2,4-D son componentes vitales del medio de cultivo para caña de azúcar, mientras que la kinetina es intrascendente o quizás de efectos inhibitorios para el crecimiento del callo. La interacción de sacarosa y 2,4-D es significativa y positiva, mientras que las interacciones de sacarosa y kinetina como la de kinetina y 2,4-D son significativas y negativas.

EMASCULATION OF SUGARCANE FLOWERS: STEAM METHOD

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ABSTRACT

Sugarcane flowers were emasculated by steam using 3 temperature ranges (40° C, 45° C and 50° C), 2 durations (5 and 10 minutes) and 2 methods (self and cross). A portable steam emasculator was used.

Steam treatment at 50°C for 10 minutes was effective in emasculating sugarcane flowers without interfering with stigma receptivity and seed setting. Arrows that were cross-pollinated after the treatment, gave germination comparable to that of the unemasculated cross (control). The seedlings produced were significantly taller than the control and all self-pollinated seedlings.

All cross-pollinated arrows significantly gave better seed germination and taller seedlings than self-pollinated arrows receiving the same treatment.

INTRODUCTION

The flowers of all varieties of sugarcane are perfect with both male and female organs present in a floret. Very few sugarcane varieties are completely male sterile (Brandes and Sartoris[1]). In arrow usually regarded as safe "females", careful examination often reveals that few anthers dehisce and give some pollen.

Most of the sugarcane varieties grown today are produced from seeds through controlled crosses using "male" and female" varieties. Although it is an improvement from the previous method of propagation from chance-pollinated arrows, there is the possibility of selfing. When a clonal variety of sugarcane is self-pollinated, the progeny appear to be very weak (Hayes *et al*[2]).

Emasculation is the removal of the anther from a flower to be used as the female variety prior to artificial cross-pollination. It may also mean rendering the pollen grains non-functional. It is desirable in crossing two make fertile varieties without the risk of selfs appearing in the progeny. It also establishes the certainty of the parentage of seedlings produced.

Stevenson[5] emphasized the importance of a reliable and effective method of emasculating sugarcane flowers without interfering with female fertility. Without

it, the diallele analysis of parent varieties, in which reciprocal crosses are made in all possible combinations is impossible.

The emasculation of sugarcane flowers was initiated by Bovell in Barbados in 1899. Since then, various methods have been attempted but were abandoned because they were impractical and not very effective. Treatment with hot water and with certain chemical sprays hold out some hope (Stevenson[5]). In the former method, the critical range of temperature for emasculation without damage to parts of the gynaecium is very narrow while for both methods, a serious disadvantage rests on the fact that at any one time, flowers in different parts of the inflorescence are in different stages of development.

Hot water has been used to kill pollen in sorghum, rice and grasses (Poehlman[4]). The flowers are immersed in hot water with temperatures ranging from 45 to 48° C for periods varying from 1 to 10 minutes, depending upon the species. Krishnamurthi[3] used three methods of emasculating sugarcane flowers. He found out that hot water treatment damages the style as well as the pollen. Cold treatment is effective but expensive while sleeving method appears to be the best.

Inspite of the advantages that can be derived from emasculation, it is not extensively used in the present breeding programs. No method that could emasculate many arrows needed to produce commercial hybrids has been found yet. This study aims to determine the effect of steam on the emasculation of sugarcane flowers. It makes use of a portable steam emasculator that can accommodate several arrows at a time. It was conducted at La Granja Station of the Philippine Sugar Commission at La Carlota City, Philippines from September, 1978 to April, 1979.

MATERIALS AND METHODS

Cane stalks of Phil 642227 were marcotted three weeks before they were expected to flower. This variety was used in the experiment because it has high degree of maleness, it flowers profusely and it is with good combining ability.

The marcotted stalks were cut at half emergence. The open flowers were clipped before the arrows were subjected to the different emasculation treatments using a potable steam emasculator locally designed for the purpose (Fig. 1).

To operate the device, the boiler was first filled to about 1/4 full of water through the Boiler opening. Water was heated by means of a kerosene stove. As the water boils, steam was generated passing through the pipes towards the steam chamber. The perforated pipes inside the chamber assures uniform distribution of heat. The temperature reading of the steam inside the chamber was determined by means of a thermometer found at the upper part of the chamber.

The treatments included 3 temperature ranges (40° C, 45° C and 50° C),

FIGURE 1. Portable steam emasculator

2 durations (5 and 10 minutes) and 2 methods (self and cross). For every treatment, some arrows were self-pollinated while the others were cross-pollinated. Self-pollinated arrows check the effect of the treatment on the pollen while cross-pollinated arrows check the effect of the treatment on female fertility. An effective treatment would kill the pollen without damaging the female parts. This means that no self seedlings will be produced on self-pollinated arrows and that many seedlings will be produced on cross-pollinated arrows.

The control consisted of untreated (unemasculated) self-pollinated arrows and untreated cross-pollinated arrows. Phil 6559 was the male variety used.

All the female arrows were planted in plastic buckets filled with compost soil. They were watered periodically to keep them alive throughout the pollination and ripening period. The male arrows were soaked in acid solution and were changed whenever necessary.

Treated and untreated arrows were pollinated under lanterns to prevent pollen contamination. Selfing was initiated by shaking the arrows while cross-pollination was effected by tapping the male arrows. This was done early in the morning for 10 days.

The arrows were bagged and harvested when the terminal spikelets started to fall off. After thorough air-drying, they were stripped of the arrows, bulked and weighed. Each treatment was composed of 12 grams fuzz seeds which were

divided into 4 parts of 3 grams each to represent 4 replications. There were sown in seedboxes containing sterilized soil.

Estimate of the number of seeds per treatment was done by getting 1 gram sample and counting the number of seeds per sample.

Germination counts were made at 2 and 4 weeks after sowing. The seedlings were given the usual nursery care. Periodic observations were made on the growth of the treated and untreated seedlings. The experimental design used was factorial in completely randomized design.

RESULTS AND DISCUSSION

Results showed that seed germination was influenced by time 2 duration x method interaction (Table 1). Consistent decrease in seed germination was noted as the degree of temperature was raised on self-pollinated seedlings except those treated at 40° C for 10 minutes. This treatment gave the highest seed germination of both the self-pollinated and cross-pollinated arrows. It was followed by treatment at 40° C for 5 minutes.

TABLE 1. Seed germination of treated and untreated arrows per 3.0 gram seeds[1].

Treatment		Seed germination[2]
40°C	5 minutes self	23.50 e
40°C	5 minutes cross	110.75 c
40°C	10 minutes self	51.25 d
40°C	10 minutes cross	177.50 a
45°C	5 minutes self	100.00 efg
45°C	5 minutes cross	106.25 c
45°C	10 minutes self	4.50 fg
45°C	10 minutes cross	42.50 d
50°C	5 minutes self	3.50 fg
50°C	5 minutes cross	58.75 d
50°C	10 minutes self	0 g
50°C	10 minutes cross	148.25 b
Untreated self (control)		19.75 ef
Untreated cross (control)		147.75 b

[1] *Approximately 4,150 seeds.*
[2] *Means not followed by the same letter are significantly different at the 5% level by Duncan's Multiple Range Test.*

Treatment with steam at 50°C for 10 minutes produced no self seedlings but the number of cross-pollinated seedlings were comparable to the untreated bi-parental cross (control). The treatment was effective in killing the pollen without damaging the female parts.

FIGURE 2a. Seedlings from self-pollinated and cross-pollinated arrows treated at 40°C for 5 minutes.

FIGURE 2b. Seedlings from self-pollinated and cross-pollinated arrows treated at 40°C for 10 minutes.

Comparable number of self and cross seedlings were observed on arrows treated at 45°C for 10 minutes and those treated at 50° C for 5 minutes. Although both treatments yielded few self seedlings, the number of cross-pollinated seedlings were significantly lower than the control.

Observations on the germination and growth of seedlings showed that seedlings on all treatments germinated at the same rate. However, all cross-pollinated arrows significantly gave better germination and seedling growth than self pollinated arrows receiving the same treatment (Fig. 2a-2g)

Measurements on plant height taken 3 months after sowing showed that except for seedlings treated at 45°C for 10 minutes, cross-pollinated seedlings in each treatment were sinigficantly taller than self-pollinated seedlings (Fig. 3). Cross-polinated arrows treated at 50°C for 10 minutes produced the tallest seedlings. They were significantly taller than the control.

FIGURE 2c. Seedlings from self-pollinated and cross-pollinated arrows treated at 45°C for 5 minutes.

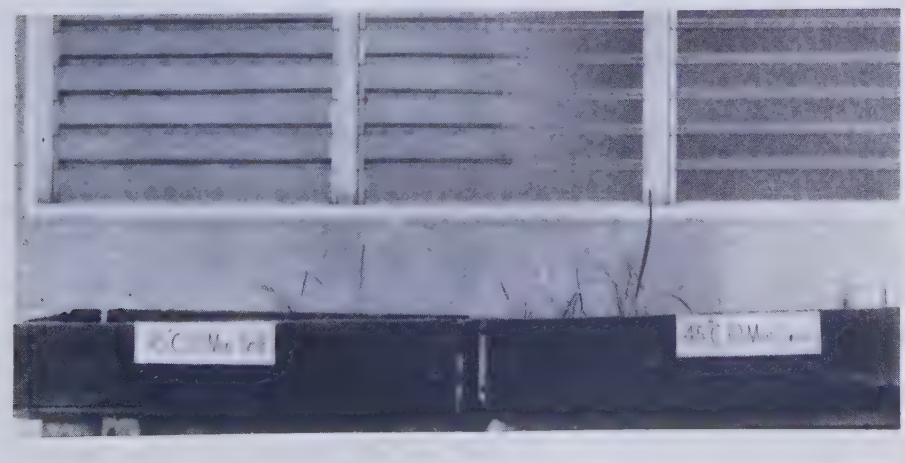

FIGURE 2d. Seedlings from self-pollinated and cross-pollinated arrows treated at 45°C for 10 minutes.

FIGURE 2e. Seedlings from self-pollinated and cross-pollinated arrows treated at 50°C for 5 minutes.

FIGURE 2f. Seedlings from self-pollinated cross-pollinated arrows treated at 50°C for 10 minutes.

FIGURE 2g. Seedlings from unemasculated, self-pollinated arrows and unemasculated cross-pollinated arrows (control).

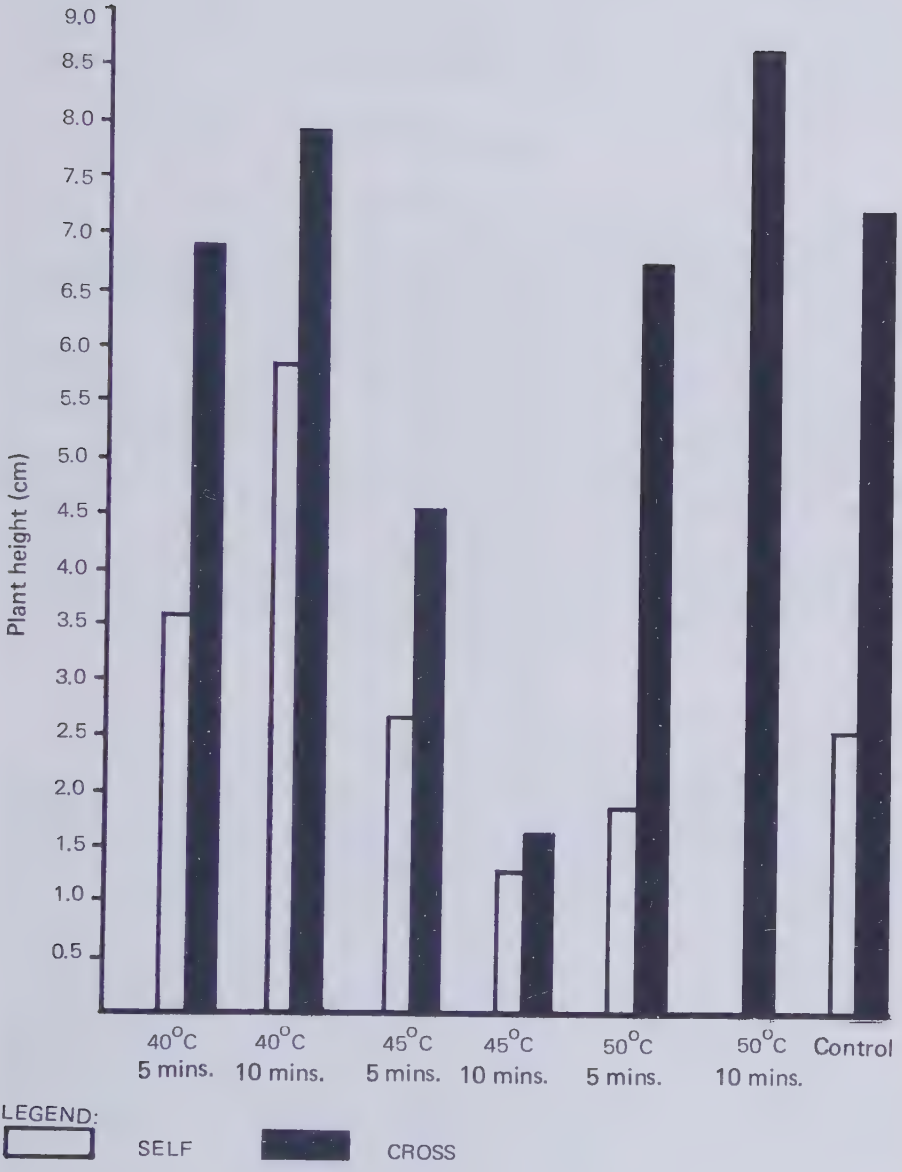

FIGURE 3. Average plant height of self and cross seedling 3 months after sowing.

CONCLUSION

Results from this study indicated that treatment with steam at 50°C for 10 minutes was effective in emasculating sugarcane flowers. The treatment killed the pollen grains without interfering with stigma receptivity and seed setting. The number of seedlings produced was comparable to the untreated cross but was significantly taller and more vigorous.

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LA EMASCULACION DE FLORES DE CAÑA DE AZUCAR POR EL METODO DE VAPOR

N.S. Divinagracia

RESUMEN

Se emascularon flores de caña de azucar por medio de vapor en 3 ranges de temperaturas (40°C, 45°C y 50°C), dos tiempos de tratamiento (5 y 10 minutos) y dos metodos/de mejoramiento (autofecundacion y cruza). Se utilizó un emasculador a vapor portatil.

El tratamiento de vapor a 50°C por 10 minutos, fue efectivo para emascular flores de caña de azucar sin interferir con la receptividad del estigma y con la formacion de semilia. Las inflorecencias utilizadas para cruzamientos despues de la emasculacion con vapor, dieron germinaciones equivalentes a las de cruzas no emasculadas (controles). Los plantines producidos ademas fueron significativamente mas altos que en los controles y que los provenientes de autofecundaciones.

En todos los casos los cruzamientos dieron mejores germinaciones y plantulas mas altas que las autofecundaciones en funcion de paridad en los tratamientos recibidos.

RELATIONSHIP BETWEEN FLOWERING AND SOME CHARACTERS IN DIFFERENT SUGAR CANE VARIETIES

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ABSTRACT

Relationship between flowering and some morphological and physiological characters in some sugarcane varieties was studied. Phenotypic correlations like cane weight, length, diameter, number of internodes and T.S.S. were studied in the different varieties. There are no significant differences in the characters between the varities which flowered and those which did not flower. Three groups were made of the varietal responses: those which flowered, those that reached some stages of flowering but could not continue, and those which did not flower. Data showed positive correlation between all characters in varieties which flowered. Two correlation were significant viz, cane weight and cane length, and cane diameter and number of internodes. Negative correlation was more in the group which did not flower.

INTRODUCTION

Sugarcane is the most important crop in Egypt for sugar production. Breeding programs here are done by importing the cuttings from abroad and selecting varieties with better quality, high yield, and good morphological characters. Research for sugar cane flowering under Egyptian conditions has been carried out since 1970. The first success was attained in 1971 – 1972 when 16 varieties flowered at Sabaheya Research Station, Alexandria (Rao, Nour, and El-Manhaly[13]). Some of them flowered early, other flowered intermediate, and some were late in flowering.

Physiological and morphological characters for some sugarcane varieties which reached the flowering stages had been studied as to their association with yield and sugar content as basis for selection. Hebert[7] obtained a significant positive association between cane yield and stalk weight, but not with cane diameter in selected clones. Legendre[9] found that cane yield was positively associated with stalk number, diameter, length, and weight. Skinner[14] studied the relationship of these important characters. Loupe *et al*[10] determined the association of stalk number, stalk diameter, erectness, and brix among and within plant-cane and first ratoon seedlings, and plant cane and first ratoon of clones derived from those

seedlings by vegetative propagation. Hebert and Henderson[8] studied stalk number, diameter, erectness, brix, and sucrose in first ratoon seedlings, and in plant cane and first ratoon of clones derived from those seedlings. Other workers (Breaux and Fanguy[1], Brown *et al*[2]). Coleman *et al*[3], George[4], Luna[11]) have studied the association of characters in first ratoon seedlings or clones.

In Trinidad, yield and sugar content are used to calculate the original profitability (Ogier[12]). George[5], investigated physiological characters as aids to selection in sugar cane.

The present study was carried out to know which characters are related with yield and sugar content, and to study the phenotypic correlation between the characters under investigation.

MATERIALS AND METHODS

Relationship between flowering and some characters in fifteen sugar cane clones from different sources under Egyptian conditions were studied. The data of this research were taken in 1977 – 1978, at the Sabaheya Research Station, Alexandria, A.R.E. The data on initiation, development, flag, tip, and full flowering stages were recorded as an average of all canes of each stage in each variety. The morphological characters like cane weight, diamater, length, and number of internodes, and T.S.S. were noted at harvesting time in July 1978. An average of 10 cane stalks for each variety was taken. The first three morphological characters were measured and T.S.S. were read with the hand refractometer.

Statistical analysis was carried out as follows:

a. Analysis of variance for each character in the different five groups and "F" value were noted.

b. Correlation coefficients between all the characters in three groups were studied. The first group contains 6 varieties which reached full flowering, the second group has 6 varieties which initiated flowering but could not continue to full stage, and the third group has the varieties which did not flower at all.

RESULTS AND DISCUSSION

Table 1 shows the dates of different flowering stages. Initiation stage occurred in October and November, flag stage in December except one variety, B 59-62 which reached it in February, tip stage was in January and February and full flowering stage extended to March. The morphological characters and T.S.S. are furnished in the same table.

Table 2 shows the average of each character for each group. There were no significant differences between the five groups for all characters.

TABLE 1. The sugar cane varieties, their flowering dates and other characters.

No.	Varieties*	Initiation date	Develop-ment date	Flag date	Tip date	Full date	Cane weight (kg.)	Cane length (cm.)	Cane diameter (cm.)	No. of inter-nodes	T.S.S.
1.	I.A.C. 52-150	31-20-77	24-11-77	20-12-77	4-2-78	4-3-78	0.60	199.5	1.6	14	18.0
2.	Col	31-10-77	24-11-77	15-12-77	16-2-78	19-3-78	1.40	282.0	2.2	10	21.6
3.	Co. 421	31-10-77	15-11-77	21-12-77	16-1-78	5-2-78	0.666	187.4	1.7	16	19.8
4.	Co. 301	30-10-77	9-11-77	12-12-77	11-1-78	4-2-78	0.714	178.6	2.0	16	12.2
5.	C.P. 52-68	30-10-77	9-11-77	12-12-77	23-1-78	22-2-78	0.600	189.4	1.9	14	15.7
6.	N.G. 292	31-10-77	18-11-77	21-12-77	14-2-78	4-3-78	0.714	191.4	2.1	19	19.7
7.	B. 59-62	31-10-77	14-11-77	4-2-78	—	—	1.250	206.8	2.3	17	20.4
8.	Bo. 34	15-11-77	28-11-77	12-12-77	—	—	1.050	226.0	1.9	1.9	18.3
9.	I.A.C. 55-29	31-10-77	24-11-77	21-12-77	—	—	1.000	312	2.1	11	18.6
10.	Co. 214	31-10-77	—	—	—	—	0.571	192.1	1.7	17	20.1
11.	Co. 469	14-11-77	—	—	—	—	1.250	212.5	1.83	20	17.7
12.	Co. 171	31-10-77	—	—	—	—	1.000	218.8	2.4	14	19.9
13.	Co. 434	—	—	—	—	—	0.600	214.2	1.7	17	16.8
14.	C0. 284	—	—	—	—	—	0.500	212.5	1.5	19	21.1
15.	Bo. 11	—	—	—	—	—	1.000	183.1	1.7	14	20.9

**The varieties from 1-3 had reached to full flowering, and produced fertile seeds.*
The varieties from 4-6 had reached to full flowering and did not give fertile seeds.
The varieties from 7-9 had reached only to flag stage.
The varieties from 10-12 had reached only to initiation stage.
The varieties from 13-15 did not reach initiation on stage.

TABLE 2. Relationship between flowering and other characters in different sugarcane varieties.

No. of group	Cane weight (k.g.)	Cane Length (cm.)	Cane diameter (cm.)	No. of internodes	T.S.S.
1	0.889	223	18	17	19.8
2	0.676	186.5	2.5	16	18.9
3	1.100	248.3	2.1	15	19.1
4	0.940	207.8	1.9	17	19.2
5	0.700	203.3	1.6	17	19.6
F value	0.7	1.68	2.6	0.19	0.09

* *1 the varieties which reached full flowering and produced fertile seeds.*
2 full flowering and did not give fertile seeds.
3 reached only up to flag stage.
4 reached only up to initiation stage.
5 varieties which did not flower at all.

Table 3 shows the correlation coefficient between all characters in the three groups of canes. All correlation coefficients were positive in varieties which reached full flowering stage. Two of these correlations were significant. In the non-flowering varieties, seven (7) negative correlations were observed and three (3) were positive in this group. In the varieties that did not reach full flowering the number of positive and negative correlation was evenly observed.

Table 3. Correlation studies between some characters of sugar cane in different varieties.

Characters	In varieties which reach full flowering	In varieties did not reach full flowering	In non-flowering varieties
	r	r	r
Between cane weight and:			
cane length	+0.95**	+0.12	–0.97
Cane diameter	+0.23	+0.45	+0.50
No. of internodes	+0.27	+0.23	–0.98
T.S.S.	+0.21	–0.36	+0.31
Between cane length and:			
cane diamater	+0.51	+0.20	–0.53
No. of internodes	+0.66	–0.77	+0.90
T.S.S.	+0.42	–0.38	–0.51
Between cane diameter and:			
No. of internodes	+0.86*	–0.50	–0.81
T.S.S.	+0.50	–0.43	–0.53
Between No. of internodes and T.S.S.	+0.65	–0.23	–0.07
d.f. = 4		d.f. = 1	
r at 0.05 = 0.811		r at 0.05 = 0.999	
r at 0.01 = 0.917		r at 0.01 = 1.000	

* *Significant*
** *Highly significant.*

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ASOCIACIONES ENTRE FLORECIMIENTO Y ALGUNOS CARACTERES EN DIFERENTES VARIEDADES DE CAÑA DE AZUCAR

A. Nour, M. El-Manhaly, R. Eskandar y S. Ibrahim

RESUMEN

La relacion entre la floracion y algunos caracteres morfologicos y fisiologicos en algunas variedades de caña de azucar, fue motivo de una investigacion en la Estacion Experimental de Sabaheya. Alejandria ARE. Se estudiaron las correlaciones fenotipicas entre peso de caña, longitud, diametro, numero de internodios y T.S.S. No se encontraron diferencias significativas entre las variedades que florecen y aquellas que no florecen. En cuanto a la floracion, las variedades pueden separarse en tres grupos: las que florecen, las que alcanzan algunos estados de floracion pero no pueden continuar y las que no florecen. Los datos muestran correlaciones positivas entre todos los caracteres en las variedades que florecen. Dos de estas correlaciones fueron significativas: peso de caña y longitud, diametro de cañas y numero de intermodios. Se encontraron correlaciones negativas en el grupo de variedades que no florecen.

STUDIES ON FLOWERING OF SUGAR CANE IN THE SOUTH OF HAINAN, CHINA

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ABSTRACT

Suinan, in the south of Hainan Island is suitable for sugarcane breeding. The place however, also has less rainy days resulting to lack of rainfall and low humidity which prevents many hard flowering varieties from flowering. Chungshen is another locality in the south of Hainan Island. Unlike Suinan, it has higher rainfall, more rainy days and higher humidity. For comparison, two hundred sugarcane varieties were planted in both places. Throughout more than ten years of experimentation and observations, flower initiation was found to be better in Chungshen than in Suinan. Fertility of pollens and settings of seeds were however, not different in both places. Even then, Chungshen is still a more ideal breeding place.

INTRODUCTION

Flowering is a process of interest to sugarcane breeders. The viability of the fuzz produced after fertilization is determined by many factors directly or indirectly influencing the process. Coleman[2] found that in Hawaii a higher minimum temperature, less hours of sunlight and higher rainfall are important factors for floral initiation.

EXPERIMENTAL PROCEDURES

World germplasms of *Saccharum* and its "relatives" were collected at the start of the study. Among the two hundred clones collected, twenty of varieties Black Cheribon, Badila and POJ 3016 did not flower in Suinan. Many of varieties such as CO 290, CO 419 and F108 (Tw.C. 108) flowered, but produced little or no viable pollens. Some (such as of varieties NCO 310) produced pollens of high sterility. Those producing high percentage of viable pollens, were mostly used as male parents, but their flowering were unstable. Therefore a need on one hand

to study flowering habits of the sugarcane varieties so as to control them as when we want them to flower, and on the other hand to locate new breeding place to compensate the defect of Suinan,are established.

Two hundred varieties of sugarcane were planted in Suinan and Chungshen. The different factors affecting the flowering of the crops were studied in both places.

RESULTS

Different Factors Affecting Sugargane Flowering

1. Moisture, rainfall and rainy days

F 134 (Tw.C. 134) was used in the study as an experimental material. In 1963, it did not flower in Suinan. It flowered however, in Suinan Chuen where the soil was more moist. As indicated in Table 1, those planted in the moist fields flowered freely, while those planted in the dry fields did not. An irrigation experiment was conducted to test the relationship of soil moisture and the flowering of sugarcane. The results, showed that weekly irrigation gave a 50.0 % flowering. Irrigating once every two week caused 41.5 % flowering while no irrigation gave no flowers at all. Irrigating F 134 was effective but for hard flowering varieties, it is ineffective. The application of a misting spray made Badila flowered at 15.5 %, POJ 3016 at 0.5 % and Yellow Caledonia at 30.0 %.

2. Nitrogen content

It is well known that nitrogen fertilizer reduces sugarcane flowering remarkably. In this experiment, sulphate of ammonia applied at 0, 90, 150 and 240 kg/acre gave 5, 18, 10 and 0 % tasseling respectively.

TABLE 1. Moisture (%) of the flowering cane fields in Suinan Chuen.

	Chuen 1	Chuen 2	Chuen 3
Flowering	13.41	9.34	8.80
Non Flowering	9.70	4.88	8.23

From a series of irrigation experiments in 1964-1967, it was observed that water holding capacity of over 60 % was the best during the floral initiating period.

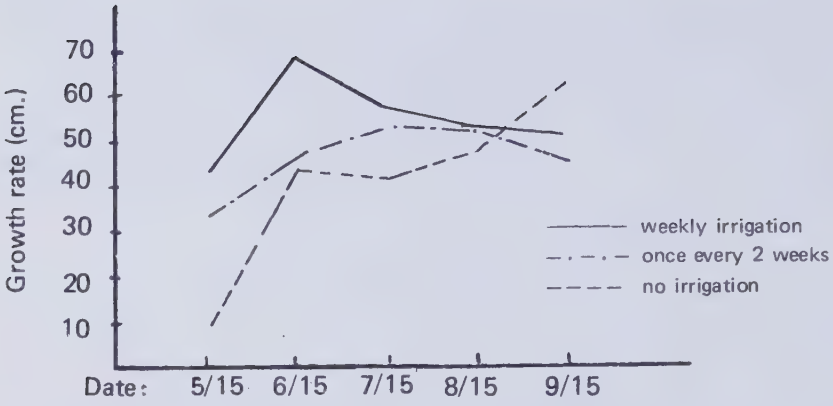

FIGURE 1. Effect of different periods of irrigation on the growth of sugarcane.

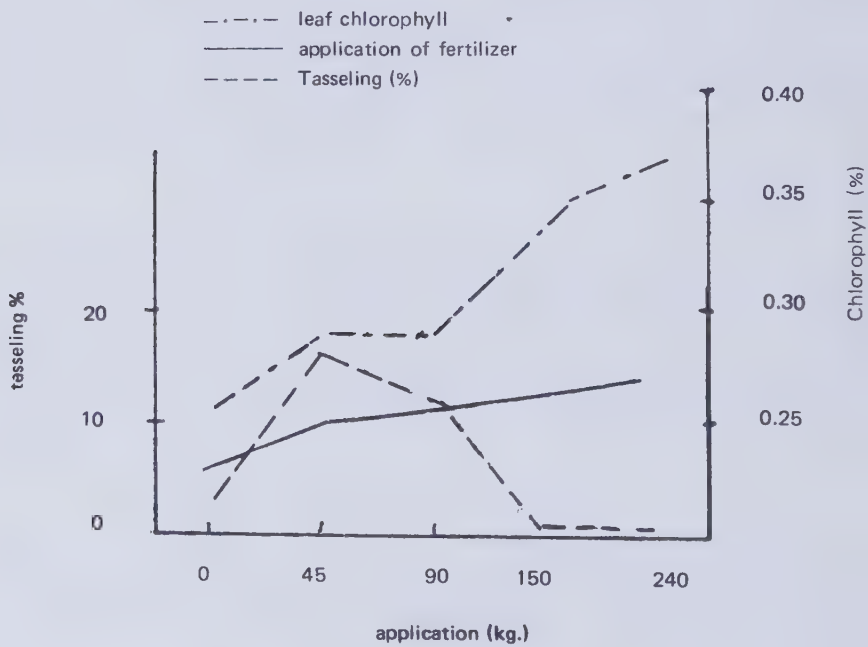

FIGURE 2. Relationship between leaf chlorophyll and the nitrogen content upon the effect of tasseling of sugarcane

3. Photoperiodism

An experiment on photoperiodism was conducted at Suinan beginning July 13 to September 9, 1964. Varieties used for the experiments were CP 49/50, POJ 3016, and F 134. Each variety was planted in a large porcelain pot, placed on carts and rolled in and out the photoperiod house every day with photoperiod control at 12 hours and 25 minutes as required for flowering of sugarcane. The results in Table 2 showed that only CP 49/50 initiated flowering from November 18 to 21, with 50 % of arrowing while the other two varieties gave no response. This illustrated that in Suinan, light is not a limiting factor for flowering of sugar cane.

4. Temperature and others

In 1965, varieties of Black Cheribon, Fiji, Crystalina, POJ 3016. Yellow Caledonia, Loethers and Striped Cheribon were planted in both places. The results showed that in Suinan, none of varieties had any sign of flowering but in Chungshen, all varieties flowered freely. Temperature and moisture conditions in both localities where some factors were considered to explain these results. As shown in Table 2, the average temperature, average minimum temperature and average maximum temperature in August, September and October were higher in Suinan than in Chungshen. The relative humidity, rainfall and rainy days however, were higher in Chungshen than in the Suinan. In these two tropical places, it seems that temperature is not a limiting factor for flowering of sugarcane while relative humidity, rainfall and rainy days are.

The same varieties in these two places were also planted for observation of fertility of pollens, the results were not significant.

To test the setting of seeds, the same combinations of parents were planted in both places. The results were presented in Table 3, showing that there was no difference between them.

TABLE 2. Temperature relative humidity, rainfall and rainy days in Suinan and Chungshen, 1965.

	Av. temp. (°C)		Av. Max. temp. (°C)		Av. Min. temp. (°C)		Relative humidity (%)		Rainfall (mm.)		Rainy days	
	S	C	S	C	S	C	S	C	S	C	S	C
Aug.	27.5	25.2	32.0	80.9	23.9	22.0	84	86	133.0	219.0	10	18
Sept.	26.0	24.0	31.3	30.0	22.9	21.1	22.9	21.1	62.2	164.9	12	15
Oct.	25.8	23.2	31.3	29.5	21.3	19.6	84	86	53.2	161.9	9	14

S = Suinan *C = Chungshen*

TABLE 3. The percentage of germination of seeds of Suinan and Chungshen.

Year	Parentage	Breeding house (%)		Outside (%)
		S	C	C
1967	CP 33/310 x F 134	2.1	6.9	3.8
	CP 49/50 x F 134	4.2	1.6	4.8
1968	CP 33/310 x F 134	4.3	4.1	1.7
	CP 49/50 x F 134	4.0	1.2	5.4
	CO419 x F 134	52.1		29.0
	NCO 310 x F 134	11.3	13.8	8.0

S = Suinan *C = Chungshen*

CONCLUSION

For more than 10 years of observations and experiments, it was found that Suinan is a suitable place for sugarcane breeding. Many hard flowering varieties however, do not flower in the locality due to lack of rainfall and lower humidity. To promote flowering of these varieties, irrigation may be necessary.

Chungshen is better than Suinan because it is situated at the foot of a mountain faces the south, is 335 meters above sea level and it has higher rainfall, more rainy days and higher relative humidity.

The morning temperature in Chungshen as compared with Suinan, is a little lower although it rises after a short period of time. The fertility of pollens and setting of seeds in the two places were not different from one another. These showed that short and low morning temperature period had no effect at all on the fertility of pollens and setting of seeds.

The varieties not flowering in Suinan, like the noble cane Black Cheribon and Crystalina, cannot be used as parents. This is a restriction in Suinan for selection of parents. At present however, this problem is already solved.

These varieties can be planted in Chungshen where they can flower freely and hybridization can be carried out directly or the arrowed stalks can be cut off and transferred to Suinan for hybridization with any selected parents. Today 30% of our crossing tassels in Suinan are transported in Chungshen every year.

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ESTUDIOS SOBRE LA FLORACION DE LA CAÑA DE AZUCAR EN EL SUR DE HAINAN, CHINA

Wong Kai Yeu

RESUMEN

Suinan se encuentra en el sud de la isla de Hainan, lugar adecuado para el mejoramiento de caña de azucar, pero con el defecto de falta de lluvias; con menos dias de lluvia y baja humedad, muchas variedades de dificil floracion no llegan a florecer en estas condiciones.

Chungshen esta en el sud de la isla de Hainan pero con mayor precipitacion, un mayor numero de dias de lluvia y alta humedad. A traves de mas de 10 años de observaciones y experimentos se encontró que la floracion es mejor en Chungshen que en Suinan. La fertilidad del polen y el desarrollo de las semillas no es sin embargo diferente en estas localidades. Esto prueba que Chungshen es una localidad ideal para establecer el programa de floracion con miras al mejoramiento genetico.

LEAF REMOVAL AS A MEANS OF DELAYING FLOWERING IN SUGARCANE BREEDING

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ABSTRACT

Six varieties with early- and heavy-flowering habits were employed in three experiments in evaluating the effectiveness of leaf removal as a means of delaying tassel production in sugarcane breeding.

It was found that the most critical factors with reference to determining an optimum date for leaf removal are the date of floral initiation and the floral initiation pattern of the variety involved. Optimum results for delaying tassels in the variety NCo 310 were produced by the absence of leaf ranks - 1 and younger for a period of 10 days. The 10-day interval must begin from the seventh to tenth day after floral initiation has taken place. Specific stages during tassel development responding to leaf removal effects on flowering delay, and the potential usefulness of the leaf-trimming technique in a sugarcane breeding program, are discussed.

INTRODUCTION

It is commonly accepted that in order to increase genetic variability in sugarcane progeny a greater range of parental combinations is necessary. However, due to the distinct flowering habits of parental clones it is often impossible for breeders to make the crosses needed to assure such diversification. For instance, early-flowering clones of *S. spontaneum* hybrids (F_1 and BC_1) often complete the flowering process by the end of October in Puerto Rico, while mid-season flowering and late-flowering parental clones begin flowering by mid-November and early-to mid-December, respectively. Consequently, crosses among these groups can rarely be made under natural environmental conditions.

Coleman[5] concluded from his work on sugarcane flowering that night light interruption during the normal floral initiation period had delayed flowering of early-flowering varieties for crossing with varieties that normally flower late. James[6] reported that, when three early-flowering clones were subjected to 12.5 hr photo-periods from September 13 to October 25, flowering was delayed up to 11 weeks and the clones could be crossed with all mid-season and many late-season flowering varieties. James and Smith[7] found that increasing the duration of a 12.5 hr photo-period from 0 to 12 weeks resulted in an increased delay of panicle

emergence. Four weeks of this treatment caused a maximum delay when it was initiated while the inflorescences were just beginning to form, or were up to 1.24 cm in length.

Moore and Heinz[9] reported that flowering of early-tasseling *Saccharum* hybrids subjected to combinations of pre-inductive and post-inductive photoperiods was delayed sufficiently to synchronize the emergence of their tassels with those of late-flowering *Saccharum* hybrids.

Julien[8] concluded from leaf-trimming experiments that removal of leaf ranks +1, +2, or the leaf spindle, delayed flowering while the removal of mature leaves ranks +3 and +4) hastened flowering in the variety U S 48-34.

Previous reports by Chu and Serapion[1,2,3,4] have shown that within the leaf spindle, the expanding leaves or the leaves just fully expanded (leaf ranks-1 and 0), appear to be most effective in producing a flowering stimulus. Absence of these young leaves during a critical period resulted in a significant reduction in flowering intensity and a delay in flowering in the variety NCo 310.

The studies presented herein were designed to evaluate the effectiveness of leaf removal with different early-flowering varieties as a means of delaying tassel production in a sugarcane breeding program.

MATERIALS & METHODS

Three leaf removal experiments were conducted during the years 1976 through 1978 at the Gurabo Substation of the UPR, Agricultural Experiment Station, Gurabo, Puerto Rico. The varieties B 50112, PR 62-195, PR 66-2314, PR 68-1058, NCo 310, and US 67-22-2 were propagated in the field and managed as reported previously (Chu and Serapion[2]). Pertinent information for three experiments is summarized in Table 1.

All suckers were removed prior to initiating treatments in each of the three experiments. Stem apices of three stalks of each test variety were sampled daily to determine the date of floral initiation. These were dissected and examined under the microscope. Tassel counts were recorded at 3-day intervals dating from the first appearance of a visible inflorescence. Stalks failing to produce a tassel were dissected in mid-January of the following year and their flowering status was recorded. A randomized block design with six replicates of each treatment was adopted for the second experiment. The replicated data for floral initiation and emerged tassels were subjected to statistical analysis.

RESULTS

Experiment 1

Flowering responses to leaf removal are presented in Table 2 and Fig. 1 to 4. Treatment effects were determined in relation to floral initiation, revert-

TABLE 1. Summary of materials and methods employed in three foliar trimming experiments

Experiment	Variety	Treatment		Number of replicates	Plot size (ft^2)	Number of stalks treated
		Leaf-rank[1/] removed	Date of removal			
1	B 50112 PR 62-195 PR 66-2314 PR 68-1058	0 and younger	A. Sept. 9. 1976	1	5.5′ x 30	
			B. Control	1	5.5′ x 30	
2	NCo 310	-1 and younger	A. Aug. 22, 1977	6	5.5′ x 8′	228
			B. Aug. 26	"	"	290
			C. (1) Aug. 30,[2/]	"	"	271
			(2) Sept. 3	"	"	
			D. (1) Sept. 3	"	"	271
			(2) Sept. 7			
			E. (1) Sept. 7	"	"	321
			(2) Sept. 11			
			F. (1) Sept. 11	"	"	251
			(2) Sept. 15			
			G. Control	"	"	289
3	US 67-22-2	-1 and younger	A. (1) Aug. 20, 1978	1	5.5′ x 8′	39
			(2) Aug. 25			
			B. Control	1	"	34

1/ The leaf having the highest visible dewlap is designated as leaf +1.

2/ The second leaf trimming was to remove the regrowth of leaf ranks −1 and younger following the first leaf removal.

ed and abortive embryonic inflorescenes, emerged inflorescences, the date of emergence, and the non-emerged inflorescences.

As indicated in Table, 2 the removal of leaf ranks 0 and younger on September 9 produced no apparent difference in initiation intensity for the varieties B 50112 and PR 68-1058. Nonetheless, this treatment produced somewhat lower intensities of flower emergence. For the varieties PR 62-195 and PR 66-2314, the same treatment caused intermediate and severe repression in floral initiation and tassel emergence, respectively. In addition, the incidences of abortive and reverted embryonic inflorescences, and of unemerged tassels, were sharply increased in these two varieties.

With reference to the time of flowering, the effects of foliar trimming on

flowering delay varied among varieties for time periods ranging from 3 to 6 weeks (Figs. 1 to 4).

Experiment 2

The initial date of floral induction for the variety NCo 310 was found to be September 1. The effects of leaf removal on flowering induction are presented in Figs. 5 and 6.

TABLE 2. Effects of leaf removal on flowering in four heavy- and early-flowering sugarcane varieties.

Variety	Treatment	Stalks treated	Date of floral initiation	Stalks initiated		Emerged inflorescences		Abortive Reverted inflorescences		Non-emerged inflorescences	
		No.		No.	%	No.	%	No.	%	No.	%
B 50112	Leaves trimmed	149	Sept. 3, 1976	125	83.9	105	70.5	10	6.7	10	6.7
	Control	163	"	135	82.8	131	80.4	3	1.8	1	0.6
PR 62-195	Leaves trimmed	145	Sept. 6, 1976	97	66.9	40	27.6	26	17.9	31	21.4
	Control	145	"	115	79.3	109	752	4	2.8	2	1.4
PR 66-2314	Leaves trimmed	186	Sept. 12, 1976	145	78.0	28	15.1	78	41.9	39	21.0
	Control	156	"	122	78.2	134	85.9	9	5.8	2	1.3
PR 68-1058	Leaves trimmed	146	Sept. 3, 1976	122	83.6	88	60.3	16	11.0	18	12.3
	Control	148	"	127	85.8	121	81.8	2	1.4	4	2.7

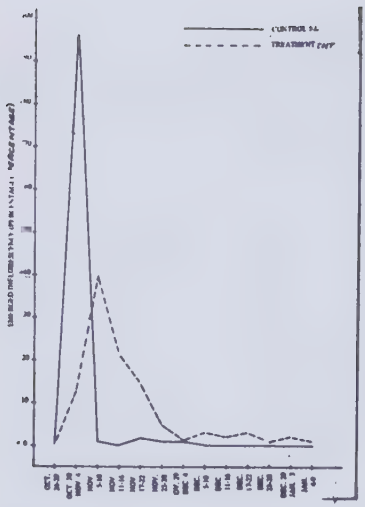

FIGURE 1. Effects of trimming leaf ranks 0 and younger on the number of tassels emerging per 6-day interval expressed as a percentage on the total number of emerged tassels in the variety B 50112.

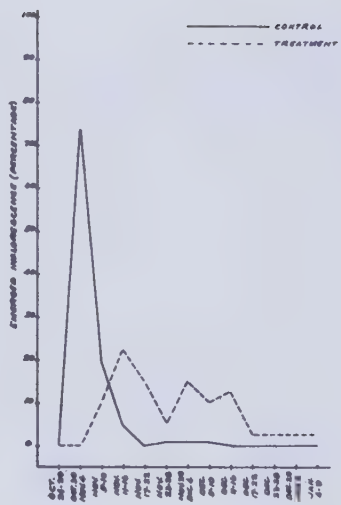

FIGURE 2. Effects of trimming leaf ranks 0 and younger on the number of tassels emerging per 6-day class interval expressed as a percentage on the total number of emerged tassels in the variety PR 62-195;

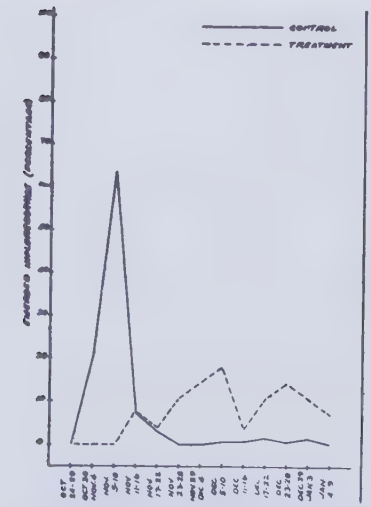

FIGURE 3. Effects of trimming leaf ranks 0 and younger on the number of tassels emerging per 6-day class interval expressed as a percentage on the total number of emerged tassels in the variety PR 66-2314.

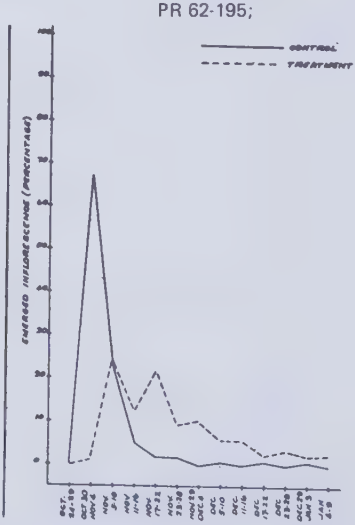

FIGURE 4. Effects of trimming leaf ranks 0 and younger on the number of tassels emerging per 6-day class interval expressed as a percentage on the total number of emerged tassels in the variety PR 68-1058.

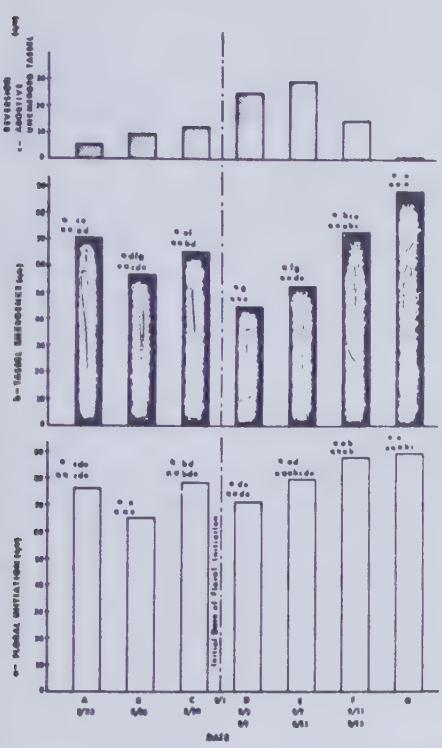

FIGURE 5. Effects of trimming leaf ranks -1 and younger on flowering in the variety NCo 310. Mean values as illustrated in bar with one or more letters in common do not differ significantly at the 5% level marked with 1 asterisk and the 1% level marked with 2 asterisks.

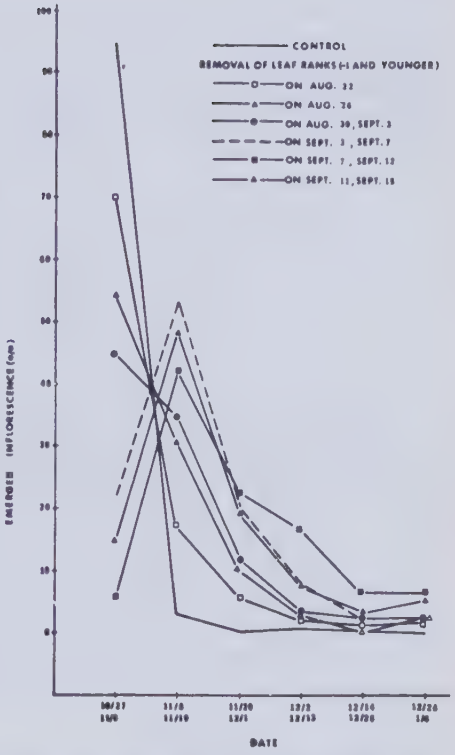

FIGURE 6. Effects of trimming leaf ranks -1 and younger on the number of tassels emerging per 12-day class interval expressed as a percentage on the total number of emerged tassels in the variety NCo 310.

As illustrated in Fig. 5-a, the removal of leaf ranks-1 and younger once on August 22 (A), and on August 26 (B) and twice on August 30, September 3 (C), and on September 3 and 7 (D), produced a significantly lower initiation intensity as compared with the control. However, none of the differences in initiation intensity among treatments, A, B C, and D were statistically significant (except between B and C).

With reference to tassel emergence, the differences between each of four treatments (A, B, C, and D) and the control were statistically significant (Fig. 5-b). Removal of the same leaf ranks at two intervals, on September 3 and 7 in treatment D and on September 7 and 11 in treatment E, caused a severe repression of tassel

emergence. This was indicated by pronounced increases in the incidence of reverted, abortive, and unemerged inflorescences (Fig. 5-c). However, treatment F (leaf ranks -1 and younger removed twice, on September 11 and 15) produced significantly lower tassel counts only at the 5% level, as compared with the control.

Greater effects of foliar trimming on flowering delay was seen in treatment E, and the least effect was produced by treatment A. the flowering dates of about three percent of the tassels in treatment E were delayed for 6 to 7 weeks; reduction were also obtained in the order of three percent for 5 weeks, 17 percent for three weeks, and 22 percent for two weeks, as compared with the control.

Experiment 3

The initial date of floral initiation for the clone U.S 67-22-2, employed in Experiment 3 was found to be August 13. The effects of trimming leaf ranks -1 and younger at two intervals (on August 20 and 25) are presented in Fig. 7. The first tassel in the control plot was visible on October 15; and the last on November 1. In the leaf removal plot the first tassel emerged on November 13 and the last on December 13 (Fig. 7). The flowering dates of the treated canes were delayed in the order of 4 to 6 weeks. As a result, crosses of this early-flowering clone, a BC_1 hybrid of *S. spontaneum,* could possibly be made with intermediate-and late-flowering varieties (Table 3).

TABLE 3. Crosses of the early-flowering clone US 67-22-2, with intermediate- and late-flowering canes, made possible by synchronizing the time of tassel emergence.

	Parental Clones		
Date of Crossing	Female		Male
Nov. 13, 1978	F 160	x	US 67-22-2
Nov. 17, 1978	PR 68-330	x	"
Nov. 21, 1978	PR 70-3391	x	"
Nov. 28, 1978	PR 70-3364	x	"
Dec. 5, 1978	PR 67-245	x	"
Dec. 13, 1978	PR 67-1070	x	"

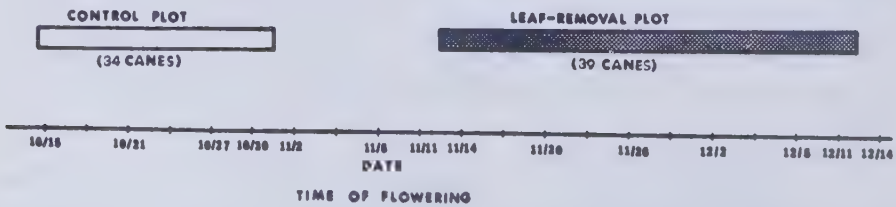

FIGURE 7. Effects of removing leaf-ranks -1 and younger on flowering in the clone US 67-22-2.

DISCUSSION

The present results are in close agreement with earlier findings in which young expending leaves, or the leaf just fully expanded, play a predominant role in the flowering processes of sugarcane (Chu and Serapion[1,2,3,4]).

It is important to note that the greatest delay of flowering was seen in the variety PR 66-2314, which had only a 15.1% tassel emergence, whereas the least delay in flowering occurred in the variety B 50112, which produced 70.5% tassel emergence (Table 2 and Figs. 1 and 4). This indicates that a large delay in flowering was associated with a low percentage of flowering. It is of further interest to note that the flowering delay for varieties B 50112 and PR 68-1058, each of which had initiated on September 3 (Table 2), was considerably different (Figs. 3 and 4). This suggests that the distinct floral initiation pattern of two varieties could be the explanation. For instance, the floral initiation period of two varieties was often seen quite different though the initial date of floral initiation for the same two varieties was found to be the same.

Little information is available concerning the specific date at which leaf removal should be performed in order to assure the greatest delay of flowering without appreciable reduction in flowering intensity. Data such as that illustrated in Fig. 5-a, b and c apparently offer the critical information which is needed for flowering delay by means of leaf removal. The results showed that removal of leaf ranks -1 and younger after September 3 had no effect on initiation (Fig. 5-a); however, continuous removal produced a significant adverse effect on tassel emergence (Fig. 5-b) and tassel development (Fig. 5-c) after September 11. This means that the effect of foliar trimming applied at sometime after September 3, approximately three days following floral initiation, for the most part, should be on development of tassels in NCo 310. The projected goal designed to obtain higher tassel emergence intensities with concurrent flowering delay, was virtually achieved by maintaining the absence of leaf ranks -1 and younger for some 10 days, beginning on the seventh or tenth day following floral initiation (Fig. 5-b and Fig. 6).

Microscopic observations of NCo 310, during the first 7-day period following floral initiation, indicated that a majority of young tassels included inflorescences with spirally-arranged meristematic patches (primary branched primordia) and an undifferentiated upper region. This finding together with the fact that significant higher tassel intensity (Fig. 5-b) and less extent in flowering delay (Fig. 6) occurring in the treatment F, as compared with the treatment E, suggests that the tassels in the process of initiating primary branch primordia, or those at the early elongation stage of primary branch primordia, are the ones most affected in flowering delay by leaf removal.

All data from the three experiments suggest that the foliar trimming technique can be practically and effectively employed as a means of delaying flowering for early-flowering varieties in a sugarcane breeding program, if it is applied with the knowledge critical to the leaf-trimming technique.

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ESTUDIOS SOBRE EL EFECTO DE ELIMINAR LAS HOJAS COMO MEDIO PARA RETRASAR LA FLORACION EN UN PROGRAMA DE MEJORAMIENTO GENETICO EN CAÑA DE AZUCAR

Teh-Ling Chu and J. L. Serapion

RESUMEN

Se utilizaron seis variedades de habito de floracion temprano y profuso en tres experimentos para evaluar la efectividad de la remocion de las hojas como medio para retrasar la produccion de inflorecencias para el programa de mejoramiento de caña de azúcar.

Se encontró que los factores mas criticos con referencia al momento optimo para la remoción de hojas son: (a) la fecha de iniciacion floral, y (b), la modalidad de la iniciacion de la floracion en la

variedad en cuestion. Se lograron optimós resultados para retrasar la emergencia floral en la variedad NCo 310 con la ausencia de las hojas -1 y mas jovenes por un periodo de 10 dias. Este periodo de 10 dias debe comenzar entre el septimó al decimo dia despues de la iniciacion floral. Se discuten en esta comunicacion los estadios especificos de desarrollo de la inflorecencia en que se encuentra respuesta a la remocion de hojas, y la utilidad potencial de la tecnica del podado de hojas para el programa de mejoramiento genetico en caña de azucar.

FURTHER STUDIES ON LEAF FLAVONOIDS AS EVOLUTIONARY INDICATORS IN *SACCHARUM OFFICINARUM* L.

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ABSTRACT

Additional data confirm that noble cane, *S. officinarum*, is characterized by a very low incidence of Tx, a high frequency of T7GS, T7GG and F9 and the absence of F4 flavonoid markers.

Possible genetic sources of these flavonoids include: Tx, T7GS and F9: *Saccharum spontaneum* (Indonesia/New Guinea forms) T7GG: *Miscanthus sinensis, Miscanthus sp.* and *Ripidum elephantinum* Absence F4: *Saccharum sanguineum, Ripidium ravennae* and *R. elephantinum.*

The combined data suggest: (1) that *S. officinarum* evolved in the Indonesia/New Guinea area rather than India (2) the hypothesis of a two step evolution of *S. officinarum* via 2n = 60 *S. robustum* is feasible and (3) the flavonoid patterns of some 2n = 60 *S. robustum* and *S. robustum* (2n = 80) are consistent with them being directly involved in the origin of *S. officinarum.*

INTRODUCTION

In previous studies [4,5,10] leaf flavonoids have been used as chemotaxonomic markers to investigate the taxonomy and evolution of sugarcane and related genera. This work indicated that some groups of clones classified under *Saccharum robustum* Brandes and Jeswiet *ex* Grassl, *Saccharum spontaneum* L, *Miscanthus* Anderss., *Ripidium* Trin. And *Sclerostachya* (Anderss.) A. Camus, could be involved in the pathways of evolution that led to the noble sugarcane *Saccharum officinarum* L.

The objective of this paper is to provide additional data to characterize more fully noble sugarcane flavonoids and investigate possible sources in related genera and *S. spontaneum.*

No additional data are provided for *Saccharum barberi* Jesw., *Saccharum sinense* Roxb. and *Saccharum robustum* as these have been subjects of recent major studies [5,10] However, data on these species have been summarized in Table 6 so that they can be readily included in the discussion.

MATERIALS AND METHODS

The method of separating leaf flavonoids by chromatography and recording with polaroid photography has been described by Paton, Shannon and Smith[9]

Leaf samples were obtained from the world collection of sugarcane germplasm maintained by USDA or through the courtesy of the Fiji Sugar Corporation (FSC) or from variety gardens operated by CSR Limited (CSR). The species and sources are listed below. As the majority of samples were from the USDA only those from other sources are indicated in the tables of results.

Species	*Sources*
Saccharum officinarum L.	15 clones selected for diversity (USDA, CSR)
Saccharum spontaneum L.	17 clones (USDA, FSC and CSR)
Ripidium arundinaceum (Retz.) Grassl	3 clones (USDA)
Ripidium bengalense (Retz.) Grassl	3 clones (USDA)
Ripidium kanashiroi (Ohwi) Grassl)	5 clones (USDA)
Ripidium procerum (Roxb.) Grassl	6 clones (USDA)
Ripidium ravennae (L.) Tin.	2 clones (USDA)
Ripidium unidentified species, samples collected in field, 1976 ISSCT expedition to Indonesia	2 clones (USDA)
Miscanthus floridulus (Labill.) Warb.	2 clones (FSC)
Miscanthus sacchariflorus (Maxim.) Benth	1 clone (USDA)
Miscanthus sinensis Anderss.	1 clone (USDA)
Miscanthus unidentified species, samples collected in field, 1976 ISSCT expedition to Indonesia and 1977 expedition to PNG	7 clones (FSC, CSR)
Imperata brasiliensis	2 clones (USDA)
Imperata exultata Brong.	3 clones (USDA)
Eulalia fastigiata Nees ex Steud.	2 clones (USDA)
Eulalia leptostachys	1 clone (USDA)
Eccoilopus hookeri (Hack.) Grassl	1 clone (USDA)
Eccoilopus longisetosus (Anderss.) Grassl	3 clones (USDA)

RESULTS

Results of this study have been combined with data obtained by Daniels, Smith, Paton & Williams[4] to present an atlas of flavonoids (Tables 1-5). In a few minor cases previous data have been revised in the light of additional results, five

TABLE 1. Leaf flavonoid patterns of *S. officinarum*

Data from Williams *et al*[12] supplemented by new data*

Unidentified flavonoids, Rf values in BAW (4:1:5) and 15% HOAC and colors in UV without and with NH_3 are

F16 70/23 dark – yellow
F17 68/07 dark – orange-yellow
F18 17/34 dark – yellow
F19 26/33 – acid yellow
F20 14/32 dark – green yellow + CSR

	Clone	Tricin					10																						
		– Tx	– 7GS	+ 7GG	+ 7RG	+ 5G	+ –	+ RG	+ TG	– IV	+ OG	– F1	+ F2	+ F3	– F4	+ F5	+ F6	– F7	– F8	– F9	– F10	– F11	– F12	– F13	– F14	– F5	– F16	– F17	– F18	– F19	– F20
	Badila	–	+	+	+	+	+	–	+	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
*	Bambu	–	+	+	+	+	+	–	–	–	+	+	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–
	Chittan	–	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–
	EK2	–	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
*	HQ409	–	–	+	+	+	+	–	+	–	+	+	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–
	Korpi	–	–	–	+	+	+	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mahona	–	+	+	+	+	+	+	+	–	+	+	+	–	–	–	+	–	–	+	–	–	–	–	+	–	–	–	–	–
*	NC30	–	+	+	+	+	–	+	+	–	+	+	–	–	–	+	+	–	–	+	–	–	–	–	+	–	–	–	–	–
*	NC32	–	+	+	+	+	+	–	–	–	+	+	–	–	–	–	+	–	–	+	–	–	–	–	+	–	–	–	–	–
*	NC53	+	+	+	+	+	+	+	–	–	+	+	–	+	–	–	+	–	–	–	–	–	+	–	+	–	–	–	–	–
*	NC64	–	+	+	+	+	–	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
*	NG21-5	–	+	+	+	+	+	–	+	–	+	+	+	–	–	+	+	–	–	+	–	–	–	–	+	–	?	–	–	–
*	NG21-6	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–
*	NG21-17	–	+	+	+	+	+	–	+	–	+	+	+	–	–	+	+	–	–	+	–	–	–	–	+	–	–	–	–	–
*	NG21-10	–	+	+	+	+	+	–	–	–	+	+	+	–	–	+	+	–	–	+	–	–	–	–	+	–	–	–	–	–
*	NG21-11	–	+	+	+	+	–	–	–	–	+	+	–	–	–	+	+	–	–	+	–	–	–	–	+	–	–	–	–	–
*	NG28-288	–	+	+	+	+	–	–	+	–	+	+	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–
*	NG51-163	–	+	+	+	+	+	+	+	–	+	+	+	–	+	–	–	+	–	–	+	–	–	–	–	+	–	–	–	–
*	NG57-76†	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	–	–	–	–	+	–	–	–	–	–
*	NG57-199	–	+	+	+	+	–	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
	NG57-225	–	+	+	+	+	+	–	–	–	+	+	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–
	Oramboo	–	+	+	+	+	+	–	–	–	–	–	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
	SC12/4	–	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–

TABLE 2. Leaf flavonoid patterns of *S. spontaneum*

Data from Daniels *et al*[4] supplemented by new data * and data from Paton *et al*[8] **

The data are arranged in ascending order of chromosome number.

When the chromosome number is not available the area of collection is noted: K = Assam; K = Kalimantan (Borneo); PNG = Papua New Guinea

The Assam clones are placed at the end of the clones from the Indian region.

			Tricin				10																						
	Chrom. No.	Clone	Tx	7GS	7GG	7RG	5G	—	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
	2n=40	SES184A	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	+	–	–	–	–	–	–
	=40	SES184B	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	–	–	–	–	–	–
*	=40	SES199B	–	–	–	+	–	+	–	–	–	+	–	–	+	+	–	+	+	–	–	–	–	+	–	+	–	–	–	–	–	–
*	=40	SES366	+	+	–	+?	–	+	+	+	–	+	+	–	+	+	–	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–
	2n=48	SES106B	–	–	–	–	+	+	+	–	–	+	+	–	+	+	+	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–
	=48	SES113A	–	–	–	+	+	+	+	+	–	–	+	–	+	+	+	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–
	2n=50	SES189	–	+	–	+?	+	+	–	+	–	–	+	+	+	+	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–
	2n=52	SES182	–	–	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–
	=52	SES186	–	–	–	+?	–	+	–	+	–	–	+	–	+	+	+	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–
	2n=54	SES154B	–	–	–	–	+	+	–	+	–	+	+	+	+	+	+	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–
*	=54	SES220	–	–	–	+?	–	+	–	+	–	+	–	–	+	+	+	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–
	=54	US4515	–	–	–	–	i	i	i	–	–	i	i	i	–	i	i	i	–	–	+	–	–	–	–	–	+	–	–	–	–	–
	2n=54	SES317	–	–	–	3?	+	+	–	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–	+	+	–	–	–	–	–	–
	=56	SES351	–	–	–	–	–	+	+	+	–	+	+	–	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
*	2n=58	SES374	–	–	–	+?	+	+	–	+	–	–	+	+	+	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–
	2n=60	SES197A	–	–	–	I?	+	+	+	+	–	+	+	+	+	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–
	=60	SES356	–	–	–	–	–	+	+	+	–	+	+	–	+	+	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
	2n=64	Coimbatore	–	–	–	–	+	+	+	–	+	+	+	+	+	+	+	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–
	=64	Mo11032	–	–	–	–	+	+	–	+	–	+	+	–	+	+	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–
	=64	SES205A	–	+	+	+?	+	+	+	+	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE 2. contd.

| Chrom. No. | Clone | Tricin | | | | 10 | | | |

TABLE 2. contd.

	Chrom. No.	Clone	Tricin					10																						
			Tx	7GS	7GG	7RG	5G	–	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
*	PNG	NG77-169	+	+	–	–	+	+	+	–	–	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	–	–	–	–	–	–
*	PNG	NG77-229	–	–	–	+	+	+	–	–	–	–	+	–	+	+	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–
	2n=96	Mandalay	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–
	=96	Tainan	–	–	–	–	+	+	+	+	+	–	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
*	2n=96	US56-4-1 †	–	–	–	+?	+	+	+	+	–	+	+	+	+	+	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–
	2n=105	Moental	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
	2n=108	Saudi Arabia	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–
	2n=112	Glagah 1286	–	–	–	–	+	+	+	+	–	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
*	2n=112	Hasuda †	–	–	–	+	+	+	–	–	–	+	+	–	+	+	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–
	=112	Okinawa	+	+	–	+	+	+	–	–	–	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
*	ñ112	Pasoeroent	–	–	–	+?	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	–	+	–	–	–	–	–	–
*	=112	Tokyo †	–	–	–	+?	–	+	+	+	–	+	+	+	+	+	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
2r	2n=126	Krakatau	–	–	–	–	+	+	+	–	–	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2n=128	M'beya	–	–	–	–	–	+	–	–	–	+	+	–	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–

FSC
† CSR
? under T7RG indicates an identification that could be T7RG of F12

TABLE 3. Leaf flavonoid patterns in *Ripidium*
Data from Daniels *et al*[4] supplemented by new data * and data from Paton *et al*[8] **

		Tricin				10																								
	Clone	Tx	7GS	7GG	7RG	5G	–	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
	R. arundinaceum																													
	Mindanao	–	–	–	+	+	+	+	+	–	–	+	–	+	+	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
	NG28-7	–	–	–	+	+	+	+	+	–	+	+	–	+	+	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
	SES7	–	–	–	+	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
	SES181	–	–	–	–	+	+	+	+	–	–	–	–	+	+	–	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–
*	Taboe Glonggong	–	–	–	+	+	+	+	+	+	–	+	+	+	+	–	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–
*	Teberau Tonggarong	–	–	–	+	+	+	–	–	–	–	+	–	+	+	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
*	US57-5-4	–	–	–	–	+	+	–	–	–	+	+	–	+	+	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
	R. bengalense																													
	IMP2888	–	–	–	+	+	+	–	+	–	+	+	+	+	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
	US47-5	–	–	–	–	+	+	+	–	–	–	+	+	+	–	+	–	–	–	+	–	–	–	+	–	+	–	–	–	–	–
	US47-14	–	–	–	+	+	+	–	+	–	–	+	–	–	–	–	+	–	+	+	–	–	–	+	–	–	–	–	–	–	–
*	US47-23	–	–	–	+	+	+	–	+	–	+	+	–	+	+	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
*	US57-10-2	–	–	–	+	+	+	–	+	–	–	+	+	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–
	US63-4-1	–	–	–	+	+	–	–	+	–	–	+	–	–	–	–	+	–	–	+	–	–	–	+	–	–	–	–	–	–	–
*	US67-9-2	–	–	–	+	+	+	–	+	–	+	+	–	–	+	+	+	–	+	–	+	–	–	+	–	–	–	–	–	–	–
*	US47-12	+	+	–	+	+	+	–	–	–	–	–	–	–	+	+	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–
	US47-18	+	+	+	+	+	+	–	–	+	+	–	–	–	–	+	+		–	–	+	–	+	–	–	–	–	–	–	–	–
	R. elephantinum																													
	SES273	–	–	+	+	+	+	–	–	–	–	+	+	–	–	+	+	–	+	+	–	–	–	+	–	–	–	–	–	–	–
	SES305	–	–	+	+	+	+	–	–	–	+	–	+	+	–	+	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–
	SES372	–	–	+	+	+	+	–	+	–	+	+	+	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–

TABLE 3. Contd.

Clone	Tricin					10																						
	Tx	7GS	7GG	7RG	5G	–	RG	TG	IV	OG	F1	F2	R3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
R. elephantinum																														
US67-14-5	–	–	+	+	+	+	–	+	–	+	+	+	–	–	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–
US67-17-1	–	–	–	+	+	+	–	+	–	+	+	+	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–
R. kanashiroi																														
* IMP 1690	–	–	–	+	+	+	–	+	+	–	+	+	+	–	+	+	–	–	+	–	–	–	+	–	–	–	–	–	–	–
IMP2384	–	–	–	–	+	+	–	+	–	+	+	–	+	+	+	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–
* IMP2385	–	–	–	–	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
* US56-12-3	–	–	–	–	+	+	–	+	+	+	+	–	–	–	–	+	–	–	–	–	–	–	+	–	–	+	–	–	–	–
* US56-12-13	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–
* US57-5-1	–	–	–	–	+	+	+	+	–	+	+	–	+	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
R. procerum																														
IMP1251	–	–	–	–	+	+	+	–	–	–	+	+	+	+	+	+	–	+	+	–	–	–	+	–	–	–	–	–	–	–
* Kalimpong	–	–	–	+	+	+	–	+	+	+	–	–	+	–	+	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–
* Mytkytina5	–	–	–	+	+	+	–	+	–	–	+	–	+	+	–	+	+	+	–	–	–	–	+	–	–	–	–	–	–	+
* SES309	–	–	–	+	+	+	–	+	–	+	–	–	+	+	–	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–
* SES311	–	–	–	+	+	+	–	+	–	+	–	–	+	–	–	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–
* SES14	–	–	–	+	+	+	–	+	–	+	+	–	+	–	–	+	+	+	–	–	–	–	+	+	–	–	–	–	–	–
* SES336	–	–	–	+	+	+	–	+	–	+	–	–	+	+	–	+	+	+	–	–	–	–	+	+	–	–	–	–	–	+
E. ravennae																														
US59-271-1	–	–	–	–	+	+	+	+	–	+	+	+	+	–	+	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–
* US59-271-2	–	–	–	–	+	+	–	+	–	+	+	–	–	–	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–
* US67-8-1 †	–	–	–	–	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+

TABLE 3. Contd.

Clone	Tricin						10																					
Ripidium species	Tx	7GS	7GG	7RG	5G	–	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
** IK76-23	–	–	–	–	+	+	–	+	–	+	+	–	+	–	–	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–
* IK76-64 †	–	–	–	–	+	+	–	+	–	–	+	–	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
* IK76-87 †	–	–	–	+	+	+	–	+	+	–	+	–	+	–	+	–	+	–	–	+	–	–	+	+	–	–	–	–	–	–
** IK76-16	–	–	–	–	+	+	–	+	–	+	+	–	+	+	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–

† CSR

TABLE 4. Leaf flavonoid patterns in *Miscanthus*
Data from Daniels *et al*[4] supplemented by new data * and data from Paton *et al*[3]

Clone	Tricin						10																					
N. floridulus	Tx	7GS	7GG	7RG	5G	–	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
ANU2057	–	–	–	–	+	+	–	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fiji 37	–	–	–	–	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Fiji 65	–	–	–	–	+	+	+	+	–	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Fiji 66	–	–	–	–	+	+	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
* Fiji 69	–	–	–	+	+	+	+	+	–	+	+	–	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
* Fiji 70	–	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
NG51-24	–	–	–	–	+	+	–	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Schodde2003	–	–	–	–	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–
US56-22-3	–	–	–	+	+	+	+	–	–	+	+	+	+	–	+	+	–	–	+	–	–	–	–	+	–	–	–	–	–	–

TABLE 4. contd.

Clone	Tricin																						
	Tx	7GS	7GG	7RG	5G	—	RG	TG	V	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
M. japonicus Henry, Formosa No. 1823A	—	—	—	—	—	+	—	—	—	+	+	+	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—
M. rufipilum US57-59-2	—	—	—	—	+	+	—	—	—	+	+	+	+	+	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—
US61-21-1	—	—	—	—	+	+	—	—	—	—	—	—	+	—	—	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—
M. sacchariflorous US64-2-2	—	—	+	+	+	—	+	—	—	+	+	+	+	+	—	+	+	—	—	—	—	—	+	—	—	—	—	—	—	+
M. sinensis Tasant T20750	—	—	—	—	+	+	+	+	+	+	+	+	—	+	+	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—
* US47-11	—	—	—	+	+	+	+	+	+	+	—	—	+	—	—	+	—	—	+	—	—	—	+	—	—	—	—	—	—	—
US59-289	—	—	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
US59-290	—	—	+	+	+	+	+	+	—	+	+	—	+	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	—
Miscanthus species (Indonesia)																														
* IS76-206 †	—	—	—	+	+	—	+	—	—	+	+	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
** IM76-240	—	—	—	+	+	—	+	—	—	+	+	—	+	—	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
** IM76-254	—	—	—	+	+	—	+	—	—	+	+	+	+	—	+	+	+	+	+	—	—	—	+	+	—	—	—	—	—	—
* IJ76-447 ¿	—	—	—	+	+	—	—	—	—	—	+	—	—	+	+	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—
* IJ76-531 ¿	—	—	—	+	+	—	+	—	—	+	+	—	+	+	—	—	—	+	—	—	—	+	—	—	—	—	—	—	—	—
Miscanthus species (PNG)																														
* NG77-22	—	—	—	+	+	—	+	—	+	+	+	+	+	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
* NG77-48	—	—	+	+	+	—	+	—	—	—	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—	—
* NG77-49	—	—	—	+	+	—	—	—	—	+	+	—	+	+	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
* NG77-56	—	—	—	+	+	—	+	—	—	+	+	+	+	+	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—

∇ FSR
+ CSR

TABLE 5. Leaf flavonoid patter in *Sclerostachya, Imperata, Eulalia* and *Eccoilopus*
Data from Daniels *et al*[4] supplemented by new data* and data from Williams *et al*[12]•••

Clone	Tricin: Tx	7GS	7GG	7RG	5G	–	10: RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
Sclerostachya fusca																														
US56-73	–	–	–	–	+	+	+	–	–	+	+	–	+	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
US56-5-2	–	–	–	–	+	+	–	+	–	+	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	+		–	–
US56-7-2	–	–	–	–	+	+	–	+	–	+	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–
US58-5-1	–	–	–	+	+	+	–	+	–	+	+	–	+	+	–	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–
US58-5-3	–	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	+	–	–	–	–	–	–
S. narenga																														
Narenga	–	–	–	–	+	+	–	–	–	+	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
US56-11-2	–	–	–	+	+	+	–	–	–	+	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–
US58-4-1	–	–	–	+	+	+	–	+	–	+	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. ridleyi																														
UM72-1	–	–	–	+	+	–	–	–	–	–	–	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Imperata brasiliensis																														
* US65-46-1	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
* US65-46-2	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–

TABLE 5. Contd.

Clone	Tricin				10																							
	Tx	7GS	7GG	7RG	5G	–	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
I. conferta																														
*** Fiji 71	–	–	–	–	+	+	+	+	–	+	+	+	+	+	–	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–
I. cylindrica																														
**	–	–	–	–	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
I. exultata																														
* US57-51-2	–	–	–	–	–	+	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
* US57-134-1	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
* US57-134-3	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
Eulalia fastigiata																														
* US62-3 3	–	–	–	–	+	+	–	–	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
* US62-3 3	–	–	–	–	+	+	–	–	–	–	–	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
E. leptostachys																														
* US57-105-3	–	–	–	–	+	+	–	–	–	–	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Eccoilopus hookeri																														
SES373	–	–	–	–	–	+	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
E. longisetosus																														
* US57-11-1	–	–	–	–	+	+	–	+	–	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–
* US57-11-2	–	–	–	–	+	+	–	–	–	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–
US57-11-3	–	–	–	–	+	+	+	–	+	–	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–

TABLE 6. Flavonoids in *S. robustum* Barberi and Sinensi Groups compared with *S. officinarum and S. spontaneum* from India and Papua New Guinea (PNG)[9]

	Tricin						10																		
S. robustum Groups	Tx	7GS	7GG	7RC	5G		RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Teboe salah	9	9	9	9	9	7	3	0	2	9	9	2	9	5	7	9	2	0	7	2	3	2	0	2	9
S. sanguineum	0	9	9	9	9	9	9	2	0	9	9	4	9	0	9	9	2	2	9	0	4	2	0	9	7
S. robustum	0	3	9	9	9	8	5	5	3	9	9	4	9	4	7	9	2	3	8	0	1	3	0	3	3
Barberi Groups																									
Mungo	0	1	9	9	8	5	1	0	0	5	9	4	5	0	1	8	1	0	0	0	0	0	0	1	0
Saretha	0	0	9	9	9	9	5	1	2	3	7	3	7	0	2	8	0	0	1	0	0	0	0	0	0
Sunnabile	0	9	9	9	9	7	3	2	0	3	7	3	7	0	0	9	0	0	0	0	0	2	0	2	0
Nargori	0	7	8	9	7	9	5	5	5	5	7	7	8	4	5	9	1	1	2	0	3	0	2	2	1
Sinense	0	8	8	9	8	9	3	0	0	3	6	6	8	3	3	9	0	0	5	0	0	0	0	0	0
S. officinarum	1	8	8	9	9	7	3	5	1	8	8	4*	4	0	5	8	0	0	6	0	0	1	0	3	0
S. spontaneum (India)	1	2	1	6?	7	9	5	7	2	8	8	4	8	9	6	8	2	1	2	2	1	3	1	4	1
S. spontaneum (PNG)	5	5	0	7?	9	9	7	4	0	9	9	6	9	9	6	9	4	0	9	0	1	2	0	2	0

[9] *Data from Daniel et al [4] and Paton et al [8] expressed on 0.9 scale where 0-completely absent 9 = always present. The data for S. officinarum and S. spontaneum have been calculated from Tables 1 and 2 respectively. Flavonoids F16-F20 have been omitted from this as their frequency is very low in this material.*

new unidentified flavonoids have been recorded: F16-F20.

Results obtained by Sinense and Barberi[10] on horticultural groups of sugarcane were compared to groups of *S. robustum*[5] (Table 6). The frequency of flavonoids in groups or species are expressed on a 0-9 scale; 0 completely absent, 9 always present.

S. robustum taxonomy is still a matter of investigation. We have followed our previous study[5] where the term *S. robustum* is reserved for the Port Moresby group (2n=80). However, we have referred to the Teboe Salah group, *Saccharum sanguineum* (Grassl), Grassl and some other clones, collectively as "2n=60 *S. robustum*" to indicate affiliation and to simplify the discussion for the general reader. The Wau-Bulolo group (2n=60) and Goroka (2n=80) group have been omitted for simplicity because they lack key flavonoids[5], have divergent morphology and cannot be directly involved in the origin of *S. officinarum.*

DISCUSSION

The objective of this discussion is to (1) define the flavonoid pattern of *S. officinarum* (2) recognize possible genetic sources of *S. officinarum* flavonoids (3) relate these possible sources to hypotheses on the origin of *S. officinarum.* No attempt is made to discuss the differences between the data of non-*Saccharum* genera and species.

Flavonoids in S. officinarum

Table 1 indicates that *S. officinarum* is characterized by a very low incidence of Tx, a high frequency of T7GS and T7GG, and absence of F4 and relatively high incidence of F9. Each flavonoid is discussed after theories of origin have been reviewed.

Hypotheses on the origin of S. officinarum

Grassl[6] has elaborated the most generally accepted theory that *S. officinarum* evolved in Indonesia/New Guinea from *S. robustum* through the agency of man. Daniels *et al* [3] extended this hypothesis and suggested that *S. robustum* (2n=80) and *S. officinarum* (2n=80) may have evolved in a two step process:

Step I: *S. spontaneum* x genus A gives 2n=60 *S. robustum*
Step 2: 2n=60 *S. robustum* x genus B gives *S. robustum* (2n-80) and *S. officinarum*

In contrast to these theories Mukerjee[7] suggests that *S. officinarum* evolved in the Assam-Bengal region from the group of related genera he called the *Saccharum* complex. Further discussion on hypotheses of origin have been reported by other researchers (Daniels, Smith & Paton[3]; Daniels, Smith, Paton & Williams[4]; Daniels, Smith, Paton & Roach[5]; and Grassl[6]).

(1) *Flavonoid Tx*

Tx is a rare tricin based flavonoid that is only prominent in *S. spontaneum* from New Guinea, the Teboe Salah 2n=60 *S. robustum* group from Indonesia and the high chromosome number of fencing clones from the New Guinea highlands [5]. The ultimate source appears to be *S. spontaneum* as Tx is present in the lowest chromosome number group 2n=40, in a clone SES 366 from Nepal (Table 2). The presence of Tx in one clone of *S. officinarum* is indicative of *S. spontaneum* involvement in the origin of *S. officinarum.* Its frequency is low probably because it does not behave dominantly in crosses (our unpublished data).

(2) *Flavonoid T7GS*

Daniels, Smith, Paton & Roach[5] indicated previously that T7GS is a marker for noble sugarcane *S. officinarum;* most nobles have T7GS and this is confirmed by data in this report. The genetic source for this flavonoid would appear to be *S. spontaneum* as it is found in a 2n=40 clone SES 366 from Nepal. However T7GS is only present in odd clones in India but achieved prominence in New Guinea *S. sponstaneum* (2n-80) with about 50% clones positive. T7GS reaches noble cane frequency in 2n=60 *S. robustum* groups in Indonesia/New Guinea with all clones of all groups being positive. T7GS is also present at noble cane frequency in *S. robustium* (2n=80).

These observations are consistent with (1) clones of *S. spontaneum* (2n=80), by chance containing T7GS, being brought by man from the Asian mainland to Indonesia/New Guinea. By this, means *S. spontaneum* in this area has a much higher frequency of T7GS than in mainland Asia, (2) two step evolution of *S. officinarum* through 2n-60 *S. robustum* where T7GS was further concentrated (3) a center of origin for *S. officinarum* in Indonesia/New Guinea where T7GS is frequent, rather than India where it is rare.

(3) *Flavonoid T7GG*

T7GG is not present in the low chromosome forms of the basic *Saccharum* species, *S. spontaneum.* We must look for the ultimate genetic source in other genera. Tables 1-5 indicate *Ripidium* and *Miscanthus.*

T7GG is prominent in *R. elephantinum* (2n=20) which has, in addition, thick stalks and lacks F4, both *S. officinarum* characteristics. If *S. officinarum* evolved in India or the Barberi sugarcane evolved independently of *S. officinarum, R. elephantinum* is a prime source of appropriate physical and biochemical characteristics.

One other odd clone of *Ripidium,* US 47-18, collected as *Ripidium bengalense* has a trace of T7GG. It is closely related to US47-12. Both these clones are clearly a typical *Ripidium* as they lack F13 which is present in all other clones of *Ripidium* and have Tx and T7GS which are absent from other *Ripidium.* This suggests that they are of another unknown genus or possible products of *Saccharum x Ripidium* introgression with a *Ripidium* phenotype. The few odd Indian higher

chromosome *S. spontaneum* which have traces of T7GG (SES205A, SES341, Dacca, SES327) are probably a complimentary group of this type of introgression but with a *S. spontaneum* phenotype.

None of the *Ripidium* clones from Indonesia/New Guinea examined to date has T7GG. However, *Miscanthus sinensis* which is present in Eastern Indonesia is a possible source. Two *Miscanthus sinensis* clones from Japan and an unidentified *Miscanthus* clone from New Guinea have strong T7GG. Unfortunately, the hairy leaved *Miscanthus sp.* presumably *M. sinensis,* collected on Sulawesi in the 1976 ISSCT expedition did not survive quarantine in Australia and was not available to us.

Sources of T7GG in the Indonesia/New Guinea area need further researching. Nevertheless a strong source is present in the area as this flavonoid is in all clones in the 2n=60 chromosome *S. sanguineum* and Teboe Salah groups and *S. robustum* (2n=80).

(4) *Flavonoid F4*

S. officinarum is characterized in these data by complete lack of F4. We are cautious in claiming complete absence because the new samples in Table 1 were obtained from plants growing under glass in the world collection, USDA Beltsville. We have found that growing cane under glass lowers the concentration of flavonoids in leaves (apart from tricins) and some minor occurrences may not have been recorded. However, the old data for *S. officinarum* with F4 absent was from field grown cane. Thus, lack of F4 appears to be a characteristic of "sugarcane" as three out of the four Barberi sub-groups also lack F4 (data from field grown cane).

This absence of F4 is of taxonomic significance as it is present in every clone of true *S. spontaneum,* the basic *Saccharum.* In addition, F4 behaves dominantly in crosses between *S. officinarum* (absent) x *S. spontaneum* (present)[12] making its absence in the Barberi clones most surprising, as it is hypothesized that Barberi has resulted from *S. officinarum* x *S. spontaneum* introgression[10]. Paton *et al*[10] suggested that the array of chromosome numbers in the Barberi groups resulted from the erosion of chromosomes from the original cross and stabilization at certain levels as basic sets of chromosome were eliminated. Perhaps the early sets which were eliminated included chromosomes with the F4 factors.

The only other genera and species to be without F4 are *Ripidium elephantinum* (2n=20) from India and 2n=60 *S. robustum* from New Guinea, principally *S. sanguineum.* They clearly cannot be the direct parents of *S. officinarum* 2n=80.

If *S. officinarum* evolved in India, or Barberi evolved independently, it is obvious that *R. elephantinum* is again a source of biochemical characteristic for a possible evolutionary pathway.

In Indonesia/New Guinea area *S. sanguineum* (2n=60) and a little known, Ramu River 2n=60 *S. robustum* group (clone US57-154-7)[5] are the only genetic source for complete lack of F4 again pointing to the importance of the 2n=60

groups. The low chromosome number *Ripidium (R. ravennae* is present in the area[2]) have a low frequency of F4. *S. sanguineum* could have arisen from introgression with *Ripidium* species in the Indonesian area. Alternatively, Price[11] found that *S. sanguineum* was a stable euploid group with associated aneuploid red fleshed "large stemmed hybrids" ranging from 2n=60-93. Perhaps *S. sanguineum* with 2n=60 is an end product after erosion of chromosomes containing the F4 factors. If an intermediate point at 2n=80 was stable, it could be the source of 2n=80 *S. robustum* and *S. officinarum.* A close relation such as this would explain why, as Price[11] has noted, in *S. sanguineum* "Old leaves and leaf sheaths fall, which exposes stalks that, perhaps more than those of any other *S. robustum* form, resemble the stalks of *S. officinarum".*

(5) *Flavonoid F9*

Attention is focused on flavonoid F9, because there appear to be significant differences and similarities between *S. officinarum* (moderate to high F9) and other groups which point to the evolution of *S. officinarum* in the Indonesia/New Guinea area.

F9 is present in most clones of *S. spontaneum,* 2n=60 *S. robustum* and *S. robustum* in the Indonesia/New Guinea region. These groups provide numerous genetic sources for F9 in sugarcane. In fact, F9 is a normal *Saccharum* flavonoid in this area.

By contrast, in India, F9 is either low or absent in *Saccharum.* It is low in *S. spontaneum* and absent or very low in the Barberi groups of sugarcane. There is a distinct difference in frequencies for F9 between India (low)and Indonesia/New Guinea (high). *S. officinarum* is clearly allied with the high group and Barberi sugarcanes with the low.

We suggest that the F9 data provide evidence for the origin of *S. officinarum* in the Indonesia/New Guinea area. It is not possible to determine whether Barberi evolved in India from low F9 *S. spontaneum* introgressing with other genera, principally *Ripidium,* or whether it is a product of introgression between *S. officinarum* (high F9) x *S. spontaneum* India (low F9). However, it is significant that the Sinense group (Pansahi) which is thought to have evolved differently *(S. officinarum* x *Miscanthus* introgression) has similar F9 frequencies to *S. officinarum.*

As a pointer for future research, we suggest that introgression between *S. spontaneum* (2n=80) from mainland Asia (low F9) x *R. ravennae* (high F9) with chromosome erosion back to 2n=80, as a possible explanation for the modification of *S. spontaneum* in the Indonesia/New Guinea area.

CONCLUSION

S. officinarum has a high frequency of clones with flavonoids T7GS and F9. T7GS and F9 are only present in high frequency in possible wild ancestors *(S. spontaneum* and *S. robustum)* in the Indonesia/New Guinea area. In India these

flavonoids do not occur simultaneously in wild species and are low in frequency. We conclude that these data support the hypothesis of Indonesia/New Guinea as the center of origin of *S. officinarum.*

This study is also compatible with the idea that the 2n=60 *S. robustum* groups could be the first step in a two step origin of *S. officinarum.* This, therefore, directs attention to these forms which have generally been neglected by breeders and evolutionists.

The general flavonoid levels in 2n=60 and 2n=80 *S. robustum* forms are consistent with evolution of *S. officinarum* from these species. These are the only wild forms discovered to date from which *S. officinarum* could have arisen directly.

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NUEVOS ESTUDIOS SOBRE LOS FLAVONOIDES EN HOJAS COMO MARCADORES QUIMIO-TAXONOMICOS PARA ESTUDIOS DE EVOLUCION EN *SACCHARUM OFFICINARUM* L.

J. Daniels, Nancy H. Patoń, P. Smith, y Christine A. Williams

RESUMEN

Información adicional permite ahora confirmar que las cañas nobles, *S. officinarum*, se caracterizan por una baja incidencia del flavonoide marcador Tx, una alta frecuencia de T7GS; T7GG y F9, y ausencia del F4.

Posibles fuentes de estos flavonoides incluyen: Tx T7GS y F9: *Saccharum spontaneum* (formas de Indonesia/Nueva Guinea). T7GG: *Miscanthus sinensis, Miscanthus* sp. y *Ripidium elephantinum.* Ausencia de F4: *Saccharum sanguineum, Ripidium ravennae* y *R. elephantinum.*

La combinacion de toda esta informacion sugiere lo siguente: (1) que *S. officinarum* evolucionó en el area de Indonesia/Nueva Guinea, más que en la India (2) La hipótesis de una evolución en 2 etapas de *S. officinarum* a traves de *S. robustum* de 2n=60 es factible (3) Los patrones flavonoides de algunos *S. robustum* 2n=60 y *S. robustum* 2n=80 son consistentes con la idea de su directa intervencion en el origen de *S. officinarum.*

THE EFFECT OF VARIOUS PLANTING DATES ON FLOWERING OF SUGARCANE VARIETIES

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ABSTRACT

Fourteen sugarcane varieties were tested for flowering in different planting seasons viz: Autumn 1977, Spring 1978, ratoon 1 1977, and ratoon 11 1978 at Sabaheya Research Station, Alexandria, A.R.E. The varieties differed in the flowering dates in each planting season.

The study showed that crossing between certain varities can be efficiently carried out by manipulating the dates of planting.

INTRODUCTIONS

For successful crossing, those varieties which are known to flower at the same time must be selected. It is well known that sugarcane varieties can be classified as early to intermediate to late flowering. Under Egyptian conditions, the varieties varied from early flowering at the second week of January to late flowering at the second week of March (Rao, Nour, and El-Mahaly[9]).

For crossing the late flowering varieties with the earlier flowering ones, the flowering dates must be modified by different treatments as controlled photoperiod (James[5,6,7]). Other authors reported flowering thru spraying some chemicals or changing the planting dates. Vijarasavadhy and Narasimham[10], Coleman[2], Daniels[3], Clements and Awada[1], George and Lalauette[4], Paliatseas[8] James[6] agree that age of cane, temperature, soil moisture, and fertility at the time of inductive day lengths interact with the photoperiod to enhance, retard, or prevent transformation of the sugarcane apices from vegetative to reproductive growth.

The present study was carried out on 14 sugar cane varieties to test their flowering behavior when planted at different seasons, viz; autumn 1977, spring 78, ratoon 1 77 and ratoon 11 78.

MATERIALS AND METHODS

Fourteen (14) varieties of sugar cane from different countries were planted at different dates, namely: autumn 1977, spring 1978, ratoon 1 1977, and ratoon II 1978. The experiment was carried out at Sabaheya Research Station, Alexandria, A.R.E. The clones were planted in 2-5 row meter length. Low rates of nitrogen

were applied 2-3 months after planting. Irrigation was carried out every 4 days. The different flowering stages were recorded and the period of every stage was noted from the first of October.

RESULTS AND DISCUSSION

Table 1 shows the dates of different flowering stages of the varieties. Autumn planting ranged from 79 days to 161 days, spring planting differed from 69 days to 168 days; ratoon I planting ranged from 83 to 221 days, and ratoon II planting differed from 84 to 161 days up to the first of October. There were also some varieties that have the same flowering date such as Co. 300, Co. 1148, and Co. 853 which flowered after 124 days. N. Co. 334 and Giza 69-54 flowered after 79 days, CP 52-68 and Co. L 48 flowered after 98 days.

The same trend was observed on other planting seasons. Those varieties which had the same flowering date can be easily crossed. Many varieties which had different dates of flowering in one season planting had the same date in another, such as the flowers of variety Co 301 after 107 days and variety GT 54-9 after 161 days. It was difficult to make crosses together, but was easy to cross varietv Co 301 from autumn planting with variety G.T. 54-9 in spring planting.

The behaviour of some varieties to reach the same stage changed by different plantings was also observed. Varieties Co. 1148 and CP. 53-1 reached to full flowering in all planting except ratoon I. This shows how the different planting can affect the flowering dates and behaviour of varieties.

Planting dates caused difference in another flowering stages. This difference ranged from 30-43, 46-61, 65-81, 90-109, and 95-125 for initiation, development, flag, tip, and full flowering, respectively.

The data show that some varieties initiated later but flowered earlier. The Indian workers concluded this when they found that the time of initiation among clones to be essentially the same regardless of time of flower emergence (Vijarasandhy, N and R Narasimhan[1] 0[10]. On the other hand, one can find relation between time of flowering and rate of development of inflorescences.

For the same variety, the different flowering stages may occur due to the environmental conditions which change from year to year.

Table 2 shows the difference in temperature in season (1977-1978) and (1978-1979), and Humidity in (1977-1978) on daily mean, and this is in turn affect the flowering initiation in different stages at different seasons. This may explain why flowering initiation changed from season to another. It also proved that temperature has a significant role in the flowering of sugar cane.

TABLE 1. Dates of flowering stages in some sugarcane varieties in different planting seasons.

No. Variety	Initiation*				Development				Flag				Tip				Full Flow			
	77 Aut.	78 Spr.	77 R I	78 R II	77 Aut.	78 Spr.	77 R I	78 R II	77 Aut.	78 Spr.	77 R I	78 R II	77 Aut.	78 Spr.	77 R I	78 R II	77 Aut.	78 Spr.	77 R I	78 R T
1. Co. 301	40	44	30	32	58	49	40	42	79	69	74	67	93	108	103	84	107	129	129	127
2. Co. 300	35	46	30	40	45	62	45	52	58	69	66	84	102	100	96	103	124	107	127	110
3. Co. 508	38	41	30	42	47	47	55	84	58	89	81	129	107	121	108	147	130	107	127	161
4. Co. 617	43	48	30	37	58	65	59	84	75	107	137	103	107	129	145	129	147	168	155	147
5. Co. 1148	46	39	30	39	47	45	45	52	58	69	–	84	98	84	–	103	124	107	–	110
6. Co. 853	40	46	30	40	56	63	52	59	79	84	127	129	102	129	138	147	124	107	155	161
7. Co. 270	46	38	38	0	73	44	55	0	147	59	660		152	67	187	0	161	67	221	161
8. N. Co. 334	32	32	30	40	39	42	38	52	49	49	45	59	60	67	73	67	79	79	103	84
9. G.T. 54–9	40	46	40	38	50	65	55	52	79	79	73	84	124	108	108	93	161	107	127	103
10. Giza 69–54	32	34	31	40	40	46	45	52	50	61	66	67	61	83	96	93	79	76	127	102
11. CP 53–1	39	0	30	39	66	0	55	59	79	0	–	93	93	0	–	103	107	0	–	110
12. CP 52–68	38	32	30	0	47	42	40	0	76	50	73	0	75	67	115	0	98	80	83	0
13. CP 54–120	39	49	30	0	56	62	66	0	66	70	137	0	89	117	145	0	136	107	154	0
14. Co. L 48	40	34	31	32	50	48	55	40	66	71	115	67	77	99	127	93	98	107	137	103
X	38.43	43	30.86	38.1	52.29	61.82	46.43	55.55	67.21	65.77	78.7	81.73	90.22	91.15	109.5	97.18	112.64	95.85	125.5	107.45

(O) = *The varieties were not included in the planting*

(c) = *The varieties which did not continue flowering*

* *All flowering stages begin from first of October.*

TABLE 2. Temperature and humidity in different seasons.

	Temperature °C				Humidity %
	1977–1978		1978–1979		1977–1978
Month	Minimum X	Maximum X	Minimum X	Maximum X	X
October	16.7	26.2	18.6	27.81	79.5
November	13.2	26.2	13.0	22.5	82.3
December	10.0	18.9	11.6	19.7	81.3
January	8.1	17.8	8.7	19.0	84.4
February	8.9	20.7	9.9	21.4	79.0
March	11.1	21.2	11.6	23.5	79.4

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DE VARIAS EPOCAS DE PLANTACION SOBRE LA FLORACION DE VARIEDADES DE CANA DE AZUCAR.

M. A. El-Manhaly, A. H. Nour, N. M. El-Talkhawy y Y. M. Tawfik

RESUMEN

Catorce variedades de caña de azucar fueron investigadas respecto a su floracion en diferentes estaciones de plantacion: otoño de 1977, primavera de 1978, primera soca 1977 y soca 1978. Las variedades fueron plantadas an epocas diferentes en cada estacion.

Puede concluirse de estas investigaciones que el programa de cruzamientos entre ciertas varie pue realizarse eficientemente escalonando la epoca de plantacion.

TRIPLOIDY IN *SACCHARUM OFFICINARUM*

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ABSTRACT

One triploid plant with 2n = 120 chromosomes was isolated from S_1 population of *Saccharum officinarum* clone 28 NG 264 (2n=80). The morphology and cytology of the triploid were studied. The triploid was more robust than the diploid. At meiosis, the triploid showed a preponderance of bivalents and univalents. Trivalents were rare. The plant was fertile on the male and female side. On selfing, the plant produced 35 seedlings which showed wide variation in morphology. The cytology of four of these seedlings were studied.

INTRODUCTION

Saccharum officinarum (2n=80) is the main source of sugar genes in the present day commercial sugarcane varieties. This species transmits diploid chromosome number when pollinated with *S. spontaneum* with rare exceptions (Bremer[3], Bull[4], Kandasami and Rao[8], Price[12]) when pollinated with *S. robustum* it transmits only haploid number of chromosomes (Kandasami[7], and Price[12]). The mechanism of the production of 2n female gamete has been investigated (Alexander[1], Bremer[2], Narayanaswamy[10]). Although *S. officinarum* produced both n and 2n types of female gametes occurrence of triploids has not been so far reported in this species. During the course of cytological analysis of selfs of *S. officinarum* clone 28 NG 264, one seedling was identified with 2n=120 chromosomes which has been found to be a triploid.

MATERIALS AND METHODS

S. officinarum clone 28 NG 264 was originally collected from New Guinea in 1928 and was being maintained by vegetative propagation in the world collection of Sugarcane Germplasm at Sugarcane Breeding Institute, Regional Research Centre, Cannanore. This clone flowers during the first week of November. It sheds profuse pollen and the fertility ranges from 60% to 70%. Two influences were self pollinated in 1973 resulting to twenty-four seedlings. One seedling was triploid with 2n=120 chromosomes. The triploid was self pollinated in 1975 and 35 seedling were obtained. Chromosome number of all the seedlings were determined from root tip cells.

For the study of meiosis spikelets at appropriate stages were fixed in 4:3:1 chloroform: Ethyl alcohol: acetic acid and later transferred and stored in 70% ethyl alcohol. Smears were made in 1% acetocarmine. Stainability of the pollen with 1:1 acetocarmine: glycerine mixture was taken as an index of pollen fertility.

RESULTS

Data collected on the morphological character of the parent (28 NG 264), its triploid S_1 and on the 35 seedlings obtained by selfing the triploid showed that with respect to plant height the triploids are superior to the diploid parent. The mean height of the parent was 195.0 cms. The triploid recorded a height of 235.0 cms and it sprogeny has a mean height of 213.5 cms, the range being 140.0 cms to 306,0 cms. Brix% was 17% in 28 NG 264, 15.8% in triploid and ranged from 13.0 to 18.2% in the seedlings of the striploid with a mean of 17.8%. No significant differences were noticed in leaf length, leaf width and stalk girth between diploids and triploids (Figs, 1, 2, 3).

FIGURE 1. *Saccharum officinarum* clone 28 NG 264 (2n=80)

FIGURE 2. *Saccharum officinarum* Triploid seedlings showing

FIGURE 3. *Saccharum officinarum* Triploid seedlings variation in morphology

Meiosis was studied in the diploid, triploid and four of the seedlings of triploid which flowered *S. officinarum* clone 28 NG 264 showed 40 bivalents at diakinesis in 20% of the cells and 39 bivalents and two univalents in 80% of the PMC's. At metaphase also 82% of the cells had 39 bivalents and two univalents and 18% of the PMC's showed 40 bivalents. The mean pairing at metaphase I was 39.30_{ii} 1.40_{i}. Chiasmata per bivalent was 1.475. Anaphase I and telophase I were normal in 65% of the cells. In 44% laggards were present. Pollen fertility of the clone was 60.80%.

In the triploid at diakinesis the number of bivalents ranged from 57 to 60 and the univalents 0 to 6. In two cells, out of 20 PMC's studied one trivalent each was noticed (Fig.4). The number of bivalents at metaphase I ranged from 56 to 60 with 0 to 8 univalents (Fig 5). Out of 20 pollen mother cells analyzed one cell showed $56_{ii}2_{iii}2_{i}$ and another had $58_{ii}4_{i}$. The mean pairing at Metaphase I was 56.6_{ii} 4.2_{i} 0.2_{iii}. Chiasmata per bivalent was 1.508. Anaphase I and telophase I had laggards ranging from 4 to 8 in 22% of the cells (Fig 6). Pollen fertility was 62.4%.

Out of the 35 seedlings obtained by selfing the triploid 28 plants showed $2n=120$ chromosome in the root tip cells. The remaining seven seedlings had chromosome numbers ranging from $2n=112$ to $2n=134$. Meiosis could be studied only in four seedlings. Out of the four seedlings which flowered, three have 120 chromosomes and one had 134 chromosomes. As in the parent, only bivalents and univalents were met with at meiosis except in one plant where one to three trivalents occurred in 20% of the PMC's. The pollen fertility of the seedlings ranged from 58.4% to 65.4%.

FIGURE 4. Metaphase I showing only bivalents and univalents in triploid

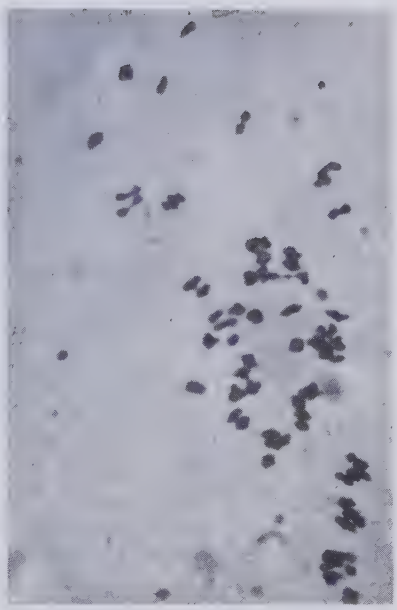

FIGURE 5. Metaphase I showing one trivalent

DISCUSSION

Modern sugarcane varieties are complex species hybrids. Although *S. officinarum* produces 2n+n hybrids in crosses with *S. spontaneum* occurrence of triploids has not been reported so far. The triploid now isolated is similar to diploid in gross morphology except for increased height of the triploid. The occurrence of a maximum number of 60 bivalents and the very rare occurrence of trivalents presents a diploid like pairing in this 'triplopolyploid'. The tendency of the chromosomes to pair only as bivalents as meiosis irrespective of the ploidy level is common in different species of *Saccharum* (Janaki Ammal[5], Panje & Babu[11], Price[12], Sreenivasan[14]). For example *S. spontaneum* has a polyploid series with chromosome numbers from 2n=40 to 2n=128. In all these forms, except for rare occurrence of one or two multivalents, only bivalents and univalents were recorded (Panje and Babu[11], Sreenivasan[14]). Janaki Ammal[5] and Kandasami[6] isolated 'triplopolyploids' from S_1 progeny of *S. spontaneum* Dacca (2=80) and Coimbatore form (2n=64). They have also observed a preponderance of bivalents, rarely univalents and multivalents.

Similar cases of diploid like chromosome pairing has been reported in many polyploid plants. Muntzing and Prakkan[9] studied the mode of chromosome paring in triploid twins of *Phleum pratense* and observed the prepondenrance of bivalents and only rare trivalents at meiosis. They have explained the results by assuming a special genotypically controlled tendency to bivalent formation. Riley and Law[13]

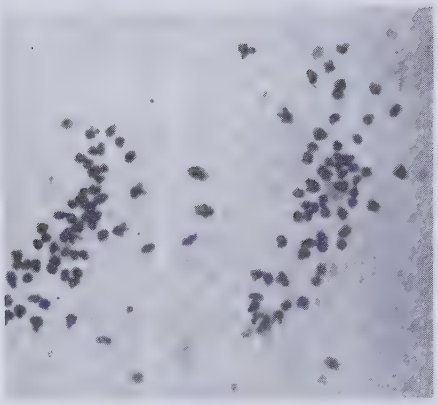

FIGURE 6. Anaphase showing laggards in triploid

have extensively reviewed the literature on genotypic control of chromosome pairing in plants. From the data available it appears that in *Saccharum* also multivalent formation is prevented and a regular meiosis is secured inspite of complete or almost complete homology between more than two genomes.

Bremer[2] had suggested that in *S. officinarum* both n and 2n types of female gametes are produced and the parthenogenetic development of female gametes with diploid chromosome number accounts for the lack of triploid progeny in selfs of *S. officinarum.* However, the present report indicates that in rare cases *S. officinarum* is capable of producing triploid seedlings.

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TRIPLOIDIA EN *SACCHARUM OFFICINARUM*

T. V. Sreenivasan y K. G. Somarajan

RESUMEN

Una planta triploide con 2n=120 cromosomas fue identificada en poblaciones S_1 del clon 28NG 264, *Saccharum officinarum* de 2n=80 cromosomas. Se investigó la morfologia y citologia de este triploide. El triploide era mas robusto que el diploide. En meiosis el triploide mostró una preponderancia de bivalentes y univalentes. Los trivalentes fueron raros. La planta mostraba fertilidad tanto como hembra que como macho. Tras autofecundar se obtuvieron 35 plantines sumamente variables en su morfologia. Se estudió tambien la citologia de cuatro de estos plantines.

THAILAND *S. SPONTANEUM* HYBRID PROGENY AS A NEW GERMPLASM SOURCE IN HAWAII

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ABSTRACT

Progeny derived from Thailand *S. spontaneum* hybridized with *S. officinarum* or commercial cultivars, have potential of becoming high tonnage commercial cultivars in the upper elevations of Hawaii.

INTRODUCTION

Crosses of sugarcane involving *S. spontaneum* with *S. officinarum* and/or commercial cultivars are an integral part of the cultivar development program in Hawaii (Heinz[5]). Since 1930, sugarcane breeders in Hawaii have hybridized over 40 different *S. spontaneum* clones with *S. officinarum* or commercial cultivars. In addition, clones of *S. spontaneum* hybridized with *S. officinarum* or commercial cultivars have been imported as vegetative cuttings (over 100) or have been germinated from seed (several hundred) imported from foreign countries.

These imported *S. spontaneum* clones or hybrids of *S. spontaneum* have been hybridized and used extensively in the Hawaiian cultivar development program.

Several sugarcane breeders have discussed the use of *S. spontaneum* clones in hybridization programs to increase genetic diversity (Dunckelman[1,2,3], Panje[9], Roach[10,11], Sankaranarayanan[12], Walker[13,14]). None have reported the derivation of new commercial hybrids from sources of *S. spontaneum* since those originally produced in Java, India and the Philippines 5 to 7 decades ago (Roach[10]). As a consequence, presently planted commercial cultivars are progeny of a very few *S. spontaneum* clones.

This paper reports the development in Hawaii of potential commercial hybrids from *S. spontaneum* clones collected in Thailand.

MATERIALS AND METHODS

The Thailand *S. spontaneums* discussed in this paper were collected as seed by Dr. A. J. Mangelsdorf between December 13-24, 1955. The basis for this 1955 expedition was a desire to collect *S. spontaneum* clones of the "Mandalay" and "Burma" types with flowering dates later than those of *S. spontaneum* already in Hawaii. Mangelsdorf had collected the Mandalay and Burma clones in 1929 in Burma, but found they flowered in October, too early for hybridizing with commercial cultivars in Hawaii. Also, under Hawaiian conditions, it was difficult to delay flowering of these two *S. spontaneum* clones. The proximity of Burma to Thailand suggested that the upper regions of Thailand might be an area containing

later flowering *S. spontaneum* and thus worthy of a collection expedition. Collections were made from 14° to 20° North latitude.

The fuzz from each collected lot was divided into three portions and sent to Hawaii, to Coimbatore, and to the USDA quarantine station, Beltsville, Maryland. The Hawaii consignment was merely for insurance. When the seedlings were established at Beltsville from all but one of 30 lots of seed, the Hawaii lot was destroyed. We have no report on the Coimbatore lot.

Seven crosses between *S. officinarum* and *S. spontaneum* were made during December, 1962 to expose dormant seed to gamma radiation. The objective was to induce translocations between *S. officinarum* and *S. spontaneum* chromosomes. Listed in Table I are the crosses, amount of "fuzz", and level of radiation. A cobalt-60 gamma source was used to irradiate the seed at levels determined in previous work (Heinz[7]).

TABLE 1. *S. officinarum X S. spontaneum* crosses, amount of fuzz (grams), radiation dosage and number of seedlings selected after sib mating.

	Dose					
	0		10KR		13KR	
Cross	Grams of fuzz	Number of sib seedlings selected	Grams of fuzz	Number of sib seedlings selected	Grams of fuzz	Number of sib seedlings selected
57NG 174[1] x US56-13-7	18.7	50	18.7	36	18.7	48
Rose Bamboo x US56-15-8	8.7	84	8.7	42		
57NG 256 x US56-16-11	17.7	0	17.7	0		
Rose Bamboo x Dacca	16.4	7	16.4	51		
28MQ 1370 x Sumatra[1]	18.2	0	8.2	0		
47NG 256 x SES84–A	16.6	5	16.6	0	16.6	0
28NG 269 x Krakatau	13.7	3	13.7	2		

[1] *57NG 174 was subsequently identified as being the same clone as Saipan 17, a Japanese developed variety widely distributed throughout the Pacific Region during World War II.*

After irradiation (January 2, 1963), the seed was germinated and hybrids were planted singly in a field at the Maunawili Breeding Station. The hybrids flowered from the 13th to the 26th of November, 1963. No treatment was given to delay flowering. Flowering hybrids within each treatment were intercrossed (Fig. 1), producing a sibbed population. Based on vigor and freedom from diseases, outstanding progeny were selected from within the sibbed populations. The progeny were asexually propagated into 1.5 x 5-meter plots under lights to delay flowering (Moore and Heinz[8]). During subsequent years, these clones were crossed to Hawaiian commercial cultivars. The resulting hybrids were distributed to regional variety selection stations representing the various environments where sugarcane is grown in Hawaii. Selected hybrids were then sent back to the breeding station for crossing with commercial cultivars, especially with those commercial cultivars proven superior in the same environment where the hybrids were selected. Then for the second time, hy-

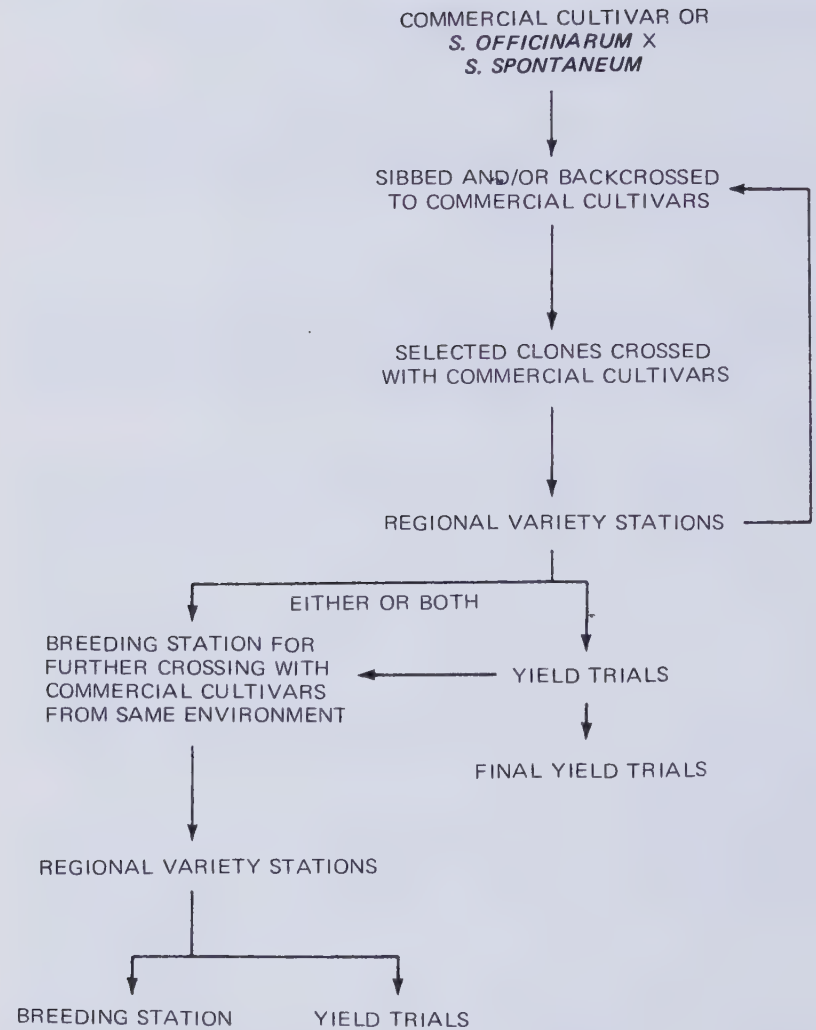


FIGURE 1. Crossing and selection scheme for development of potential commercial cultivars from *S. spontaneum.*

brids' were sent back to the regional station representing the environmental niche. They were selected and returned to the breeding station for further crossing with commercial cultivars. This occurred up to three or four generations of backcrossing to commercial types. At any stage in the cycles, hybrids could have been selected and installed into yield trials.

Parallel with the radiation study, other crosses were made involving Thailand *S. spontaneum* clones with other commercial hybrids which have produced progeny with commercial potential. Progeny from the crosses were backcrossed and selected using the procedures outlined above.

Yield trials were of two types: the preliminary (FT5) with a plot size of 9 x 7 meters and the advanced (FT70 with a plot size of 12 x 12 meters. Installation of tests and analysis of test data were according to the augmented block design by Federer[4]. Cultural practices, including nutrition, were those used on the plantations where the yield trials were grown. Chromosome counts were determined from microsporocytes and/or young leaf tissue using procedures previously outlined (Heinz *et al*[6]). Disease ratings were based on a scale of 1 to 9, 1 for most resistant and 9 for most susceptible to the disease.

RESULTS AND DISCUSSION

Irradiation

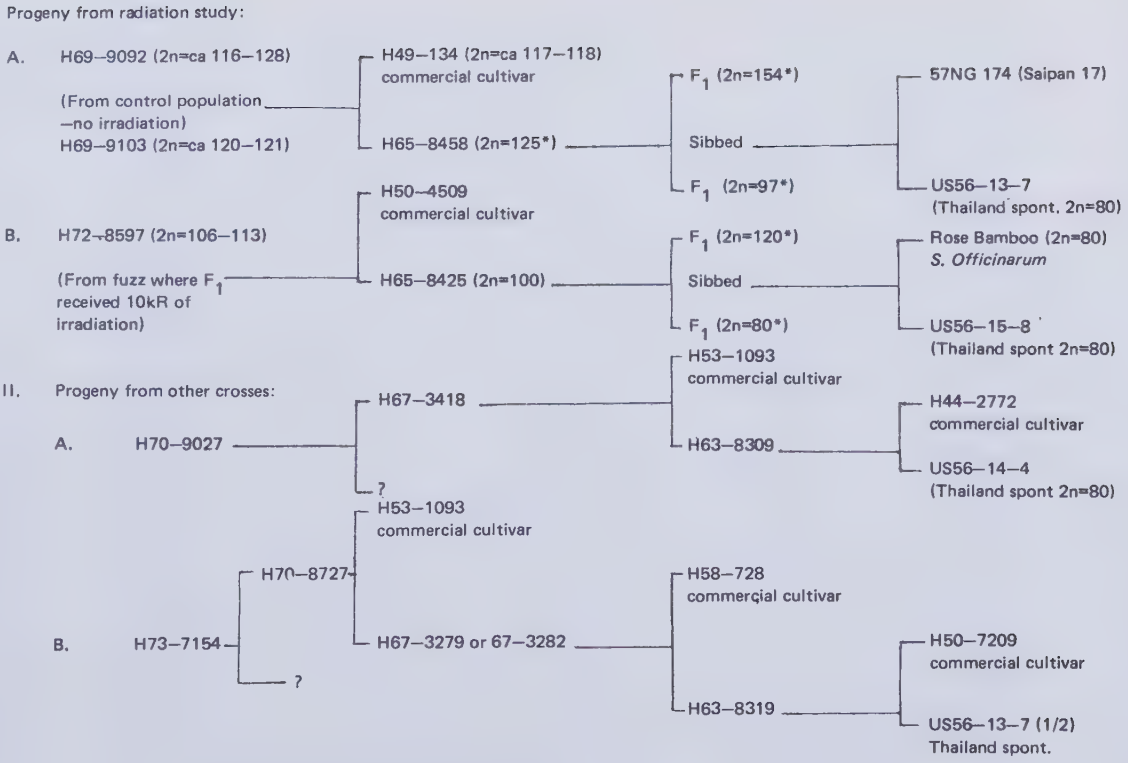
Irradiated and control populations were studied for induction of chromosomal translocations and menotypic changes. Although chromosome counts were not taken for all progeny in the irridiated populations, microsporocyte examination of up to 10 individuals in each population at diakinesis and metaphase showed no differences between irradiated and control populations. Normal bivalent pairing and no multivalent pairing were observed. Except for an initial reduction in vigor in the irradiated populations, no phenotypic differences attributable to irradiation could be identified. Between the sibbed populations, no distinguishable differences were observed. With the exception of H65-8425, chromosome counts were not taken from the progeny of the sibbed populations.

Chromosome Transmission

Chromosome numbers are available for some progeny of the crosses under discussion (Fig. 2). Consider, for example, H69-9092 and H69-9103 with chromosome numbers of 2n = ca 120, as the progeny of the commercial female parent H49-134 and the male parent, H65-8458. Since the female parent had a chromosome complement of 2n = ca 117-118, one can by inference assign a chromosome number of 2n = ca 125 for H65-8458. This chromosome number for H65-8458 is reasonable if one assumes a cross between sibs having n + n and 2n + n (2n = 154) chromosomes as the parent material. If this was the case, then a rare event has occurred in which a commercial hybrid, 57NG174 (Saipan 17) has passed on the intact full chromosome complement to its progeny. Another possible explanation for 2n = 125 in H65-8458 is contamination by foreign pollen. This was very unlikely though since the sib mating was conducted under Australian type cloth lanterns which have proven adequate to provide an environment free of foreign pollen.

A more substantive argument for 2n + n and n + n transmission is provided by H65-8425 with a chromosome number of 2n = 100 (Fig. 3), derived from a sibbed population producing H72-8597 (Fig. 2) with a chromosome number of 2n = Ca 106-113 (Fig. 4). The only missing chromosome number is for H50-4509, a Hawaiian commercial cultivar which we assume has a chromosome number of about 2n = 120.

One can only speculate on the importance of chromosome number transmission in these progeny. Nevertheless, we would like to emphasize in the case of progeny derived from Thailand *S. spontaneum*, that 2n + n transmission is a real possibility and suggests that the chromosome balance plays an important role in the yield potential of these clones.



**Chromosome number by inference.*

FIGURE 2. Pedigrees of progeny producing acceptable yields, including chromosome number when known. *S. spontaneum* line is shown in detail.

Yield Data

More important, based on our observations, is the source of the *S. spontaneum* parent materials from the 19°-20° north latitude and the elevation from which they were collected in Thailand. Evidently, these *S. spontaneums* evolved in a climate where they established great buffering capacity for they seem to be prepotent for passing on to progeny regardless of the female parent, the ability to thrive in the low sunlight upper elevations of Hawaii. Commercial parents such as H50-4509 and H50-7209 have not performed well in the low sunlight upper elevations.

Progeny from the *S. spontaneum* X commercial-type crosses were distributed to seven different ecological zones for observation and selection. Generally in the lower elevations, the progeny were spont-like, had small stalk girth, heavy stooling and were very trashy. The cultivars H69-9092, H69-9103 and H72-8597 exhibited more *S. spontaneum* characteristics at the lower elevations than they did at the higher elevations. These cultivars were vigorous, healthy in appearance and had excellent ratooning ability when grown at the higher elevations. At the lower elevations they were heavy flowering, whereas at 450 meters or higher, flowering was almost completely inhibited.

FIGURE 3. Photomicrograph showing 50 bivalents for H65-8425. As in the sibbed progeny studied no multivalent chromosome formations were detected in this clone derived from an irradiated population.

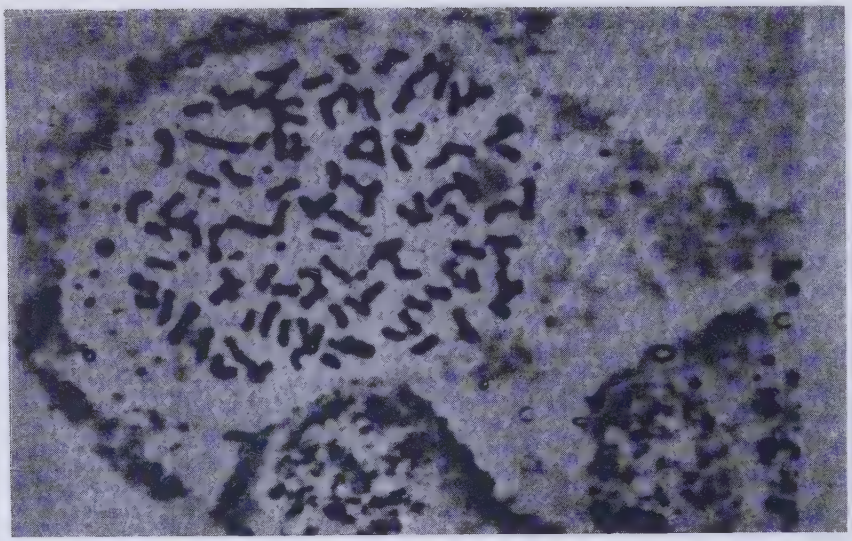


FIGURE 4. Photomicrograph of chromosomes (2n = 109) in young leaf tissue of H72-8597.

Field data reported were only for those crosses and progeny producing acceptable cane tonnage and sugar yields in FT5 and/or FT7 yield tests. Acceptable yields were obtained from four basic crosses (Fig. 2). Reported in Table 2 are yields of five cultivars showing yield potential. Clones H69-9092, H69-9103 and H72-8597 are planted in replicated tests in the upper elevations and some will be harvested in 1980. Based on their early growth, stalk number, stalk girth and stalk length up to 14 months of age, they appear to be substantially ahead of the control plots. They can produce heavy cane tonnage, but the juice quality is generally poorer than desired. However, enough data were available to suggest that these clones will perform satisfactorily in Hawaii.

The higher cane tonnage potential of these cultivars at the higher elevations of Hawaii, where long crop age is practiced, indicated that they can provide germplasm for improving yields. Based on preliminary yields, the progeny of the first backcross (BC_1) may be placed in commercial production under these conditions. With the availability of new chemical ripeners, the inherently lower sucrose content may not be such a yield-limiting factor. While one should not expect the same sucrose percentage from chemically ripened low-sucrose cultivars as one would from similarly ripened high-sucrose cultivars, the high cane tonnage of the *S. spontaneum* hybrids will give opportunity for the production of the same or higher total sugar per hectare. In addition, there will be a higher yield per hectare in total dry weight including total sugar, molasses and fiber.

The increased production of dry matter per hectare is of importance in Hawaii which is almost totally dependent on imported fuel oil for energy. It is a state government policy to encourage energy self-sufficiency. In part this will come

TABLE 2. Yield data and disease ratings of progeny showing acceptable yields in yield trials.

Cultivar	Estimated Tons Cane/HA		Estimated Sucrose Yield % Cane		Estimated Tons Sugar/HA		Harvest Age	Type of Test	Elevation	Eyespot	Disease Leaf Scald	Smut	
	Cultivar	Control	Cultivar	Control	Cultivar	Control	Months		Meters			A	B
H69-9092 [US56-13-7(BC_1)]	369	297	9.7	12.4	35.8	36.7	29.7	FT5	471	1	a	4	1
	281	209	11.9	13.7	33.5	24.5	26.7	FT7	471				
H69-9103 [US56-13-7(BC_1)]	578	493	15.3	12.8	85.7	62.6	47.9	FT5	660	1	a	2	5
	508	337	9.4	13.7	46.4	45.9	25.9	FT5	420				
	272	281	10.1	12.5	27.9	34.9	21.7	FT7	300				
	351	337	12.3	12.3	42.5	41.4	26.9	FT7	180				
H72-8597 [US56-15-8(BC_1)]	353	274	14.8	12.9	50.6	35.8	29.9	FT5	450	a	a	1	0
H70-9027 [US56-14-4(BC_3)]	349	299	12.4	14.9	43.4	44.3	35.8	FT5	465	2	2	5	8
	252	263	11.1	15.4	28.3	40.1	21.5	FT5	84				
H73-7154 [US56-13-7(BC_4)]	202	171	12.3	14.4	26.6	24.3	18.8	FT5	231	1	2	1	a

a = Rating not available

through increased biomass production. Sugarcane can provide increased energy through increased fiber production. Already on the island of Hawaii, approximately 45% of the electricity generated is from bagasse. This percentage can be increased by increasing total crop yield per hectare. The Thailand *S. spontaneum* progeny offers the opportunity to develop cultivars higher in total sugar, fiber and molasses per hectare.

Our preliminary data suggested that the Thailand *S. spontaneum* sources have the potential of producing high-yielding progeny under Hawaiian conditions. Similarly, Dunkelman[2] reported that US56-15-8 produces progeny having high yield potential in Louisiana. Although some BC_1 progeny have promising yield potential, the probability is that BC_2 or BC_3 progeny from the BC_1 cultivars will be the clones having the desired characteristics for high yielding cultivars.

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LA PROGENIE HIBRIDA DE *S. SPONTANEUM* DE TAILANDIA COMO UNA NUEVA FUENTE DE GERMAPLASMA EN HAWAII

Don. J. Heinz

RESUMEN

La progenie derivada de *S. spontaneum* de Tailandia hibridizada con *S. officinarum* o cultivares comerciales tiene capacidad potencial de brindas cultivares comerciales de altos tonelajes para las zonas mas altas de Hawaii.

EVALUATION OF SUGARCANE CLONES AND ITS RELATIONSHIPS WITH THEIR PARENTS FOR RESISTANCE TO STEMBORER

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ABSTRACT

In a field trial of sugarcane clones obtained in 1968, an evaluation of the intensity of infestation of the stemborer *Diatraea saccharalis* was made on plant cane and first ratoon including other important traits like pol and fiber % cane and reducing sugars.

The data obtained were studied in an attempt to link them with one another and with the parentage of the crosses.

It was found out that parentage did influence the results. Co 419 and IAC 52-179 showed a tendency to produce crosses with the highest degree of resistance to the stemborer. On the other hand, CB 40-69 showed a tendency to generate crosses with the highest degree of susceptibility. CB 40-35 produced crosses with a wide range of susceptibility or resistance.

The method used showed great value for the screening of the material to be utilized in a breeding program searching for resistance of sugarcane varieties to the stemborer.

INTRODUCTION

The stemborer (*Diatraea saccharalis*) is a major problem in most sugarcane areas of Brazil, alone or associated with rot diseases (Gallo *et al*[4], Planalsucar[9], Guagliumi[5], Silva and Pompeo[13]). It is a pest widely distributed from Florida to Texas (in the U.S.), Mexico, West Indies, Colombia, Guyana, Brazil to Argentina, Ecuador and Peru (Bleszynski[1]). Charpentier and Mathes[2] stated that although the injury inflicted by stalk moth borers varies in degree from one cane-growing country to another, it is often of major economic importance and that *D. saccharalis* is responsible for the greatest damage.

Mathes and Charpentier[7], after listing the type of damage caused by borer attack, stated that it lessens the amount of juice that can be extracted

from the stalks and the percentage of sucrose in this juice; the purity of the juice is correspondingly lowered; total organic non-sugars are increased; and the color of the clarified juices and syrups is darkened and their turbidity increased. This deterioration resulting from borer injury is increased by entrance of fungi and bacteria into the borer tunnels. Borer injury also retards maturation. Metcalfe[8] stated that the wealth of literature on the damage by sugarcane moth borers is indicative of their economic importance. Scaramuzza[11] stated that *Diatraea* spp were causing in America an annual loss conservatively estimated at no less than 60 million dollars.

In a sequence of our breeding program a series of crosses was made in 1968 searching for new sugarcane varieties of high cane and sugar yields allied with pest and disease resistance. This paper presents an evaluation that was made to determine the relative resistance or susceptibility of the selected clones of that series to the stemborer. It also presents the relationships between the intensity of infestation (Gallo *et al*[4]) and other main characteristics like pol and fiber % cane and reducing sugars. Further, this resistance or susceptibility deals with the parents used in the respective crosses.

MATERIALS AND METHODS

The materials included 24 *Saccharum* spp clones obtained after several years of selection and six commercial varieties namely CB 41-76, NA 56-79, IAC 51-205, IAC 52-150, IAC 58-480 and CB 47-355. These six varieties represent about 70% of the sugarcane area planted in the State of Sao Paulo (Planalsucar[10]). The clones are listed in Table 1.

One of the field experiments with the above mentioned clones was located at Barra Grande de Lencois Mill, an area which has problems with the stemborer *D. saccharalis,* and provides a high grade of natural infestation. The data presented here were obtained from sugarcane crop planted on March 3, 1976 in a dark red latosol sandy phase soil using a randomized block design with four replications.

The intensity of infestation was determined according to Gallo *et al*[4] and Guagliumi[5] after two cuttings (plant cane, Aug. 22, 1977 and first ratoon, Sept. 18, 1978). It consisted of the following: 40 stalks were taken at random and the number of joints were counted and the stalks opened longitudinally to determine the number of attacked joints. Intensity of Infestation was then determine using the formula: I = 100 B/T, where I = Intensity of Infestation, B = number of attacked joints and T = total number of joints.

Analysis of pol and fiber % cane were made according to Tanimoto[15] and reducing sugars were determined by conventional method. Laboratory determinations were made in both plant cane and first ratoon. However, just the latter ones were used to obtain the coefficients of correlation.

TABLE 1. Clones tested, their parentage, commercial varieties used and data obtained from the field trial for intensity of infestation, plant cane and first ratoon average

Parentage	Cross No.	Intensity of of Infestation %
IAC 52-179 O.P.	cl 68-69	14.79
Co 419 x IAC 52-179	cl 68-1	15.73
CB 40-35 O.P.	cl 68-127	15.97
Co 419 O.P.	cl 68-84	16.37
(Co 419 x ?)	(NA 56-79)	16.62
Co 419 x IAC 52-179	cl 68-12	16.80
(POJ 2878 x Co 213)	(CB 47-355)	16.85
CB 40-35 O.P.	cl 68-109	16.89
CB 40-35 O.P.	cl 68-120	17.73
Co 419 x IAC 52-179	cl 68-8	18.01
CB 40-35 O.P.	cl 68-130	18.03
CB 40-35 O.P.	cl 68-129	18.22
CP 34-120 x ?	cl 68-17	18.25
CB 40-35 O.P.	cl 68-113	18.26
(POJ 2878 x CP 44-101)	(IAC 58-480)	18.49
CB 40-35 O.P.	cl 68-112	18.82
(POJ 2878 x ?)	(CB 41-76)	18.95
Co 356 O.P.	cl 68-82	19.17
CB 40-35 O.P.	cl 68-135	20.18
CB 40-35 O.P.	cl 68-119	20.21
CB 40-69 O.P.	cl 68-158	20.55
IAC 52-179 O.P.	cl 68-65	20.92
(POJ 2878 x ?)	(IAC 51-205)	22.15
CB 40-69 O.P.	cl 68-153	22.40
CB 40-35 O.P.	cl 68-104	22.41
Co 419 O.P.	cl 68-88	22.60
CB 40-69 O.P.	cl 68-140	22.91
(Co 419 x Co 285)	(IAC 52-150)	23.20
IAC 51-204 O.P.	cl 68-53	23.96
CB 40-69 O.P.	cl 68-144	24.55

( ) — *Within brackets are commercial varieties*

O.P. — *Open pollination*

Means linked by a bar are not statistically different (Duncan test, 5% level).

RESULTS AND DISCUSSION

An analysis of variance was made for the data on the Intensity of Infestation in the first and second cuttings and a total analysis was carried out. For the

first cutting (plant cane) the analysis showed no differences among treatments; for the second cutting (first ratoon), F test showed significant differences among treatments at 1% level. The results showed that the Intensity of Infestation for the first cutting (27.69%) was almost three times higher than that for the second cutting (10.97%). According to Gallo *et al*[4] and Guagliumi[5], the former had a very high grade and the latter, a medium grade of infestation. This decrease in the Intensity of Infestation agrees with the results obtained by Silva and Moraes[12], Teran and Noraretti[16], and Macedo, Mendes and Botelho[6].

The total analysis has shown F test significant for the effects of treatments (5% level) and years (1% level) but the effect of interaction (treatments x years) was not significant. The treatment means for Intensity of Infestation are shown in Table 1. The treatment with the lowest Intensity of Infestation (cl 68-69) was not statistically different from that of the 22nd position in the score (cl 68-65). The grade of infestation varied from a medium (14.79%) for cl 68-69 to a high one (24.55%) for cl 68-144, considering the two cuttings.

Looking at the parentage of the clones, a close relationship between the most resistant crosses and their parents Co 419 and IAC 52-179 used in open pollination or in biparental crosses is almost evident. On the other hand, there is evidence that CB 40-69 produced the most susceptible clones. CB 40-35 produced crosses with a wide range of susceptibility or resistance.

It is interesting to note that Gallo[3] found in five cane-growing areas of the State of Sao Paulo the following average of Intensity of Infestation: 15.8%, 21.5% and 38.8%, respectively for the varieties Co 419, CB 41-76 and CB 40-69, which are in agreement with our data.

Table 2 shows the results obtained in the second cutting (first ratoon) on the Intensity of Infestation, fiber % cane, pol % cane and reducing sugars. With the grade of infestation of stemborer varying from a low (5.23%) to a high one, (20.78%) differences among treatments seem more evident. It is easy to distinguish a group of 15 treatments with a low to moderate grade of infestation and another with a medium to high grade. With a few exceptions it is easier to see the influence of the parentage on the Intensity of Infestation, confirming the above observations made in Table 1.

The Intensity of Infestation of the stemborer did not affect the industrial traits and vice-versa. However, cl 68-144 with the highest grade of infestation (20.78%) presented the lowest fiber content (9.4%). Pol % cane varied at random, from 12.94% for cl 68-153 to 17.08% for cl 68-135, both placed at the end of the rank of intensity of infestation. Similar fact occurred with the reducing sugars which varied from .22 to .54.

Linear coefficients of correlation were carried out on the data and the results of which are shown in Table 3. Intensity of Infestation was negatively correlated without statistical significance, with all the other traits and in a higher degree with fiber % cane ($r = -0.20$). This demonstrate that at least

there is a tendency of the clones that showed greater amount of fiber showed greater resistance. This result confirms the data on Mathes and Charpentier[7] who found out that r = –0.506 from studies with 27 varieties in Louisiana for these two traits. On the other hand, Silva and Moraes[12] observed that stemborer infestation together with rot diseases can effectively increase fiber %, destroying healthy tissues. It was supposed that intensity of infestation would increase reducing sugar but the coefficient of correlation observed

TABLE 2. Data obtained on intensity of infestation, fiber % cane, pol % cane reducing sugars for the second cutting (first ratoon, 1978).

Parentage	Cross No.	Intensity of Infestation %	Fiber % cane	Pol % cane	Reducing sugars
IAC 52-179 O.P.	cl 68-69	5.23	10.8	16.02	.36
CB 40-35 O.P.	cl 68-120	5.54	11.4	15.77	.54
Co 419 x IAC 52-179	cl 68-12	6.14	11.3	15.93	.24
(Co 419 x ?)	(NA 56-79)	7.12	10.4	15.82	.23
(POJ 2878 x Co 213)	(CB 47-355)	7.72	10.8	14.60	.35
Co 419 O.P.	cl 68-84	7.79	11.1	16.04	.23
CB 40-35 O.P.	cl 68-130	8.04	11.8	15.75	.30
CP 34-120 x ?	cl 68-17	8.11	12.0	16.15	.23
CB 40-35 O.P.	cl 68-127	8.41	12.2	13.76	.26
Co 419 x IAC 52-179	cl 68-1	8.67	12.0	15.90	.22
(POJ 2878 x ?)	(CB 41-76)	8.93	10.5	15.92	.26
CB 40-35 O.P.	cl 68-113	9.97	10.5	16.24	.26
CB 40-35 O.P.	cl 68-112	10.17	11.5	15.23	.31
CB 40-35 O.P.	cl 68-129	10.25	10.6	15.03	.26
Co 419 x IAC 52-179	cl 68-8	10.60	10.5	13.86	.25
CB 40-35 O.P.	cl 68-109	11.20	11.8	16.49	.22
CB 40-35 O.P.	cl 68-119	11.80	12.0	15.95	.38
IAC 51-204 O.P.	cl 68-53	11.93	12.8	14.81	.22
(POJ 2878 x CP 44-101)	(IAC 58-480)	12.46	11.7	15.90	.22
CB 40-69 O.P.	cl 68-140	12.67	11.5	14.55	.23
Co 356 O.P.	cl 68-82	12.71	11.2	15.38	.28
Co 419 O.P.	cl 68-88	12.93	11.3	14.06	.48
IAC 52-179 O.P.	cl 68-65	13.03	11.5	13.47	.44
(POJ 2878 x ?)	(IAC 51-205)	13.43	11.0	15.98	.28
CB 40-69 O.P.	cl 68-158	13.61	12.2	14.61	.23
(Co 419 x Co 285)	(IAC 52-150)	13.67	10.8	14.88	.27
CB 40-69 O.P.	cl 68-158	13.87	11.1	12.94	.44
CB 40-35 O.P.	cl 68-104	16.03	10.6	16.81	.25
CB 40-35 O.P.	cl 68-135	16.37	11.5	17.08	.22
CB 40-69 O.P.	cl 68-144	20.78	9.4	16.08	.23

( ) – *Within brackets are commercial varieties*

O.P. – *Open pollination*

in this experiment showed a tendency of negative correlation which is in agreement with the finding of Souza[14], although not statistically significant. The data on reducing sugars and pol % cane demonstrate the highest negative (and significant) value for the coefficient of correlation.

TABLE 3. Linear coefficient of correlation (r) between the studied traits of sugarcane.

	Fiber % cane	Reducing sugars	Pol % cane
Intensity of infestation *(D. saccharalis)*	- 0.20	- 0.15	- 0.04
Fiber % cane		0.02	0.14
Reducing sugars			- 0.42+

+ *t test significant at 5% level*

The results indicate the method used can be of great value for the screening of the material to be utilized in a breeding program searching for resistance to the stemborer as much as it can be used during the selection procedure itself. This is very important because in a breeding program for commercial cane varieties, Mathes and Charpentier[7] stated that it is almost as important to avoid the use of borer-susceptible parents as it is to actually use borer-resistant parents.

CONCLUSIONS

a) Co 419 and IAC 52-179 produced crosses with the highest resistance to the stemborer *Diatraea saccharalis.*

b) CB 40-69 produced crosses with the highest susceptibility to the stemborer.

c) Crosses derived from CB 40-35 presented a wide range of susceptibility or resistance to *D. saccharalis.*

d) The method used showed great value for the screening of materials to be used in a breeding program for resistance to stemborer.

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EVALUACION DE CLONES DE CAÑA DE AZUCAR Y LA RELACION CON SUS PROGENITORES EN LA RESISTENCIA AL GUSANO PERFORADOR *Diatraea saccharalis* (Fabr.)

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RESUMEN

Sobre la base de un experimento de campo con clones de caña de azucar obtenidos en 1968, se efectuo una evaluacion de la intensidad de ataque de gusano perforador *Diatraea saccharalis* en caña planta y en caña soca, ademas del control de otras importantes caracteristicas como pol y fibra % caña y azucares reductores.

Los datos obtenidos fueron estudiados en un intento por relacionarlos entre si y con la ascendencia de los clones.

Se encontro que los progenitores influenciaron los resultados. Co 419 y IAC 52-179 mostraron una tendencia a producir progenies con alto grado de resistencia al gusano perforador. Por otro lado CB 40-69 mostro una tendencia generar cruzamientos con alto grado de susceptibilidad. CB 40-35 produjo progenies con un amplio rango de respuestas desde susceptibilidad a resistencia.

El metodo utilizado mostro gran valor para la investigacion del material a ser utilizado en el mejoramiento genetico, en la busqueda de resistencia de variedades al gusano perforador.

ASSOCIATION BETWEEN SOME CHARACTERS OF SUGARCANE *(SACCHARUM SPP.)* GROWING IN FOUR LOCALITIES IN THE NORTH–EAST OF BRAZIL

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ABSTRACT

Correlations between components of yield, quality and leaf area obtained from 6 varieties of sugarcane during 3 different periods of development were done. The varieties were grown in 4 distinct regions in the North-East of Brazil. Generally, the correlations studied presented quite diversified behavior, that is, some had the same tendency for all the localities and others did not clearly present themselves equally. The latter points to an existence of genotype-environment interaction.

INTRODUCTION

The importance of choosing information about associations between characters of varieties is a well known phenomenon among sugarcane breeders. Many publications about the subject are available in relevant literature. Associations between characters like yield/plot, length, diameter, and number of stalk, pol%, fiber% in the cane and many other characters are well documented. The magnitude of these associations are peculiar to genotype populations and the environmental conditions.

Recently, great emphasis has been laid on studies on characters which influence the photosynthetic capacity like leaf area, length and width of the leaf, the number of leaves per plant and the angle of the leaf insertion on the stalk. Shimabuku[13] showed the coefficient of light extinction (k) to be an important factor in the analysis of yield components and that the values of this coefficient depend on the plant structure.

According to Shimabuku and Higa[14] the coefficient (k) presents a strong association positively identified with the index of leaf area during the initial period of the plant development, but this association is negative during the plant's period of greater development. Furthermore, the same authors show that the coefficient (k) is negatively associated with both the cane yield and sugar yields.

In the present work, results of a preliminary study are presented and these will serve as a guide for future studies on the correlations of some yield components and some characters which influence the photosynthetic capacity.

MATERIALS AND METHODS

Six sugarcane varieties with different architectures were planted in four experimental areas localized in distinct regions in the North-East of Brazil. The design employed was a completely randomized block with 3 replications. Each plot was composed of 4 rows 10 m long with an interrow spacing of 1.40 m. The varieties used were CB45-3, C0997, Co1007, RB7025, RB7075 and RB70283. The last three are clones obtained through the breeding program of PLANALSUCAR.

For leaf width and length characteristics the measurements were done during three periods. The first period constitutes the initiation of plant development, the second corresponds to the period of the plant's greater development and finally the third coincides with the harvest period. From the results thus obtained, leaf areas for the varieties were calculated during each experimental period using the following expression:

$$\text{Leaf Area} = (\text{Leaf length} \times (\text{Leaf width}) \times (0.67)$$

Width and length measurements were done on the leaf +1 corresponding to the last visible dewlop.

The measurements for the stalk length and average diameters were done during the harvesting periods of the experiments, using 10 stalks randomly picked in each plot. Pol and fiber percentages in the cane were obtained in the laboratory through the analysis of two samples from each plot.

RESULTS AND DISCUSSION

The experimental coefficients variation (CV) were considered to be low, thus indicating a good experimental precision. These are presented in Table 1 together with a general average (X) of each experiment.

Of the characters studied, the most important for increasing cane yield production seems to be the stalk length; a positive correlation was found between the two characteristics and besides presenting significance in two localities, the same tendency was observed in other localities (Table 2). This shows that a selection for the stalk length leads to an increase in cane yield.

It is important to emphasize that in spite of not having studied the characteristic of stalk numbers, it is our common knowledge that the same is of great interest in the process of selection, principally in the presentation of a strong positive association with yield according to the works of Zacarias[15], James[5]. Mariotti[11] and others.

A correlation between stalk diameter with cane yield per plot was significant only in one locality and the tendencies in the remaining localities were not uniform. However, when this character was not favorable, it did not also present direct limitations to the increase in cane yield.

TABLE 1. Coefficient of variation (CV%) and the averages of each characteristic in each locality.

Characteristics	Locality							
	Sta. Terezinha		Barreiros		O. D' agua		Estivas	
	CV%	X	CV%	X	CV%	X	CV%	X
Cane yield/plot (kg)	8.84	489.39	7.40	585.06	5.20	617.22	10.07	541.89
Leaf area (1) (cm^2)	20.82	206.60	11.83	257.02	8.10	266.82	10.87	193.37
Leaf area (2) (cm^2)	6.33	600.46	10.78	488.66	5.68	552.1	5.23	514.19
Leaf area (3) (cm^2)	6.48	474.57	7.61	474.75	4.35	522.48	7.70	426.07
Leaf width (1) (cm)	7.80	3.26	8.21	3.51	4.08	3.35	5.94	3.15
Leaf width (2) (cm)	4.71	5.08	5.69	4.69	3.55	5.65	3.48	4.86
Leaf width (3) (cm)	4.39	4.39	4.07	4.60	3.42	4.92	5.61	4.62
Leaf length (1) (cm)	14.73	92.80	6.16	108.21	7.20	118.72	8.52	91.85
Leaf length (2) (cm)	4.90	176.59	4.73	160.37	3.41	146.44	3.39	157.81
Leaf length (3) (cm)	5.53	159.09	4.42	153.72	2.55	158.36	3.52	137.63
Stalk diameter (cm)	4.63	2.46	3.35	2.54	3.14	2.81	2.81	2.52
Stalk length (cm)	6.00	273.09	4.34	285.79	1.67	322.12	7.59	291.65
Pol % in cane	6.03	13.79	5.16	16.89	4.73	15.09	4.60	16.54
Fiber % in cane	7.09	11.82	7.16	11.60	8.59	11.30	10.75	12.94

(1) – Measured during the initial of the plant's growth.
(2) – Measured in the intermediary phase.
(3) – Measured in the harvesting period.

Correlations with negative tendencies were found between pol% and cane yield per plot for all the localities; the magnitudes varied from highly significant to almost nil.

TABLE 2. The coefficients of correlation between the yield and quality components.

Associations	Locality			
	Sta. Terezinha	Barreiros	O. D'agua	Estivas
Cane yield/plot and				
Stalk diameter	0.2990	0.6711**	0.3497	–0.1014
Stalk length	0.6899**	0.5166**	0.2209	0.4296
Pol % in cane	–0.3450	–0.3789	–0.7656**	–0.1464
Fibre % in cane	–0.1961	0.1704	–0.1695	0.0319
Stalk diameter and				
Stalk length	0.5306*	0.5526*	–0.1033	0.0338
Pol % in cane	–0.1394	–0.6559**	0.1144	0.0338
Fibre % in cane	0.0980	0.2517	–0.3776	0.2268
Stalk length and				
Pol % in cane	–0.7991**	–0.5796*	–0.5914*	–0.4829*
Fibre % in cane	–0.2159	0.4059	0.3699	0.2543
Pol % in cane				
Fibre % in cane	0.1241	–0.2323	0.3699	0.2543

* *level of significance at 5%*
** *level of significance at 1%*

Practically the correlations found between the fibre% and cane yield per plot were nil, indicating that selection can be effected to increase or diminish the fiber content without affecting the yield of cane.

The correlations between the stalk diameter and its length were positive and significant for two localities and nil for the rest. However, the stalk diameter and pol% presented a highly negative correlation just for one area, and it was in this same area that the diameter played a big role in the yield increase. The length of stalk presented negative correlations with the pol% for all the localities.

In the literature available, many authors talk about this subject where great variations in the results are justified by different material and the environmental conditions of the experiment (Cesnik and Vencovsky[1], George [2,3] Herbert[4] James[5] and Falgout[6], Khairwal *et al*[7] . Mariotti [8,9,10,11], Miller and James[12], Zacarias[15]).

A positive correlation between leaf area, leaf width and length was observed

during the three periods in which the measurements were taken at all localities (Table 3).

TABLE 3. Coefficients of correlations between leaf area and other characteristics

Associations	Locality			
	S. Terezinha	Barreiros	O. D'agua	Estivas
Leaf Area (1) and				
Leaf width (1)	0.8856**	0.7966**	0.8562**	0.5144*
Leaf length (1)	0.9265**	0.6009**	0.6480**	0.7505**
Cane yield/plot	0.5106*	0.2362	0.2464	0.1588
Stalk diameter	0.4663	0.1388	-0.4722*	0.3993
Stalk length	0.3858	0.3374	0.1636	-0.2211
Pol % in cane	-0.1611	0.0838	0.2669	0.1773
Fibre % in cane	0.4954*	0.6584*	0.4273	0.1835
Leaf Area (2) and				
Leaf width (2)	0.5548*	0.7514	0.7568**	0.4384
Leaf length (2)	0.5552*	0.6009	0.8169**	0.2361
Cane yield/plot	0.6583**	0.3182	-0.1441	0.0786
Stalk diameter	0.7544**	0.2121	0.4320	0.3406
Stalk length	0.4175	0.1347	-0.2879	0.2777
Pol % in cane	-0.1455	-0.1416	0.4423	0.4687*
Fibre % in cane	-0.0427	0.1624	0.0201	0.0807
Leaf Area (3) and				
Leaf width (3)	0.7880**	0.8583**	0.8247**	0.8824**
Leaf length (3)	0.8640**	0.9498**	0.8409**	0.6843**
Cane yield/plot	0.2015	0.2046	0.2859	0.4613
Stalk diameter	0.5198*	0.5080*	0.5580*	0.0632
Stalk length	0.0064	0.3279	-0.31.03	0.0438
Pol % in cane	0.3142	-0.4320	0.4788*	0.3163
Fibre % in cane	0.0556	-0.1127	-0.1177	0.2111

(1) — Measured during the initial of the plant's growth
(2) — Measured in the intermediary phase.
(3) — Measured in the harvesting period.
* — *level of significance of 5%*
** — *level of significance at 1%*

The correlation coefficient between the leaf area and the cane yield per plot in the same locality was positive in the first and second periods, but in the third period it presented a uniform tendency in all the localities even if it was not significant. Variable results were obtained for the values of the coefficients of correlations for leaf area and the stalk diameter during the first period for each locality, but in the second and third periods, the correlations with leaf area were always positive.

One interesting observation made here was the positive correlations between leaf area and the fiber content in the first period. Here, not only was the same tendency observed for all localities but it was also seen to be significant for two of them.

It is suggested that in future studies not only should leaf area be taken into consideration alone but also the leaf area index, this being a more representative measure of the photosynthetic capacity.

The correlations considered in this study presented diversified behavior that is, the same tendency to be positive or negative can be observed generally in the localities. Others did not present similar tendencies. The latter case indicates an existence of an interaction of genotype with the environment.This phenomenon has called the attention of those breeders who tried to minimize it through ecological zonation or in choosing varieties that are more stable.

The results obtained here cannot be considered to be definite; they are just a guideline to what may happen with the characters studied in these different localities. However, they point to a necessity of considering the localities individually in later studies. These studies should have as their main objective the estimation of important parameters while determining the procedure for selection. Among these parameters, coefficients of heritability were included as well as the genetic variation and the part of each characteristic gained during selection. Furthermore, it would be important to know direct and indirect effects between these characteristics by genetic and phenotypic correlations and one analysis of coefficient of path. Materials that are representative of the germplasm must be used in the breeding program.

CONCLUSIONS

From the results presented above, the following conclusions were reached:

a) There is a tendency of positive correlations between the following characters:

Cane yield and stalk length
Leaf area during the three periods and the leaf width during the corresponding periods.
Leaf area during the three periods and the leaf length during the corresponding periods.
Leaf area during the first period and the fiber percentage.
Leaf area in the first and second periods and stalk diameter.
Leaf area in the third period and yield.

b) There is a tendency of negative correlation between the following characters:

- Cane yield and pol% in the cane.
- Stalk length and pol% in the cane.

c) The correlations between the remaining characters do not present a general tendency for the four localities, thus indicating that these localities must be individually considered in future studies.

d) Leaf area index is suggested for the best representation of photosynthetic capacity.

e) The necessity of wider experiments which permit establishment of genetic correlation and the analysis of the coefficient of path between the characters is hereby emphasized.

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ASOCIACIONES ENTRE CARACTERES DE CAÑA DE AZUCAR *(SACCHARUM SPP)* CULTIVADA EN CUATRO LOCALIDADES EN EL NORESTE DE BRASIL.

C. E. L. S. Pires y E. F. Sobral da Costa

RESUMEN

Los resultados que se presentan en esta comunicacion se refieren a correlaciones entre componentes del rendimiento, calidad y area folliar, obtenidos en seis variedades de caña de azucar en tres diferentes estados de desarrollo. Estas variedades fueron cultivadas en cuatro regiones diferentes del noreste de Brasil. En general las correlaciones presentan un comportamiento muy diverso, siendo algunas muy similares entre las localidades investigadas mientras que otras no presentan tendencias claras. Este ultimo caso senala la ocurrencia de interacciones entre genotipos y ambientes.

A REVIEW OF THE WORLD DISTRIBUTION OF *PUCCINIA* SPP ATTACKING SUGAR CANE

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ABSTRACT

Reports since 1940 on *Puccinia* spp attacking sugar cane were reviewed. In several cases, the pathogen had been identified initially as *P. kuehnii*, but was in fact, *P. melanocephala.* The identity of the pathogen should be rechecked in all countries, unless the identification was made recently.

Unless the few American reports can be confirmed, present information showed that *P. kuehnii* probably occurs only in the Asian-Australian-Pacific region. All previous African reports seemed to be erroneous.

***P. melanocephala* has a much wider distribution, and is likely to be identified in many more countries over the next few years. A modified chronology for the spread of this pathogen was proposed. New world distribution lists were presented for both pathogens.**

INTRODUCTION

There are two pathogens causing rust diseases on sugar cane (Egan[12]). *Puccinia kuehnii* Butl. has been known since the late 1800's, and is generally regarded as a weak pathogen causing a disease of very minor importance. *P. melanocephala* H. & P. Syd. (synonym *P. erianthi* Padw. and Khan) was first reported as a sugar cane pathogen in 1949 (Patel *et al.*[27]), and causes a more serious disease problem.

Rust disease of sugarcane has been reported from a number of countries on or adjacent to the African continent since the 1940s. The pathogen sometimes was identified as *P. kuehnii*, and sometimes as *P. melanocephala* (= *P. erianthi*). Yet a common pattern of varietal susceptibility could be distinguished in the Indian, African, American and Australian outbreaks (Egan[14]). The literature on these rust disease outbreaks indicated that only one pathogen seemed to be involved. It was *P. melanocephala.*

The 1978, outbreaks of sugar cane rust disease in the Caribbean (Koike *et al* [21], Presley *et al* [28]) and in Australia (Egan and Ryan[15]) prompted a re-examination to verify whether *Puccinia* sp. really was the pathogen responsible for those outbreaks.

MATERIALS AND METHODS

Using reports since 1940, the world wide distribution of *Puccinia* spp. was determined.

RESULTS AND DISCUSSION

Previous records of rust occurrence

Table 1 lists newly recorded reports on *Puccinia* sp. attacking commercial sugar cane in Asia, Africa, America and Australia since the 1940s. The list is not considered exhaustive and there are probably available references with updated and correct findings for some countries.

Based on available literature, the following information on rust outbreaks were evident.

a. *P. kuehnii* rust

This first occurred in South Africa in the early 1940s, on sugar cane variety Co301. It continued infecting Co301 and other varieties up to the early 1970s (McMartin[25], Bailey p.c.).

In the 1956 ISSCT World List of diseases (Martin[23] this rust was reported to occur in Mozambique and Malagasy Republic but the basis for this is not known. Heavy rusting in variety B4362 was noted in Malagasy Republic before 1962 (Hughes[19]). This variety was also badly affected in Cameroon in 1969 (Barat[8]). The Mauritius and Reunion rust outbreaks were considered to be due to a series of cyclones which carried rust spores from Malagasy Republic (Atonine and Perombelon[5], Hoarau[17]).

P. kuehnii was reported from the American region in Cuba (Martin[23]) Guatemala and U.S.A. (Baker *et al* [7]), and Mexico (McGuire and Crandall[24]). There are several secondary references of later dates which seem to refer back to these reports or other primary sources. It may be significant that the ISSCT Standing Committee on Sugar Cane Diseases has never shown rust as occurring in any American country except for Cuba in the 1956 list (Martin[23]). This was withdrawn in the 1959 and subsequent lists, presumably on advice from Cuba.

b. *P. melanocephala* rust

The initial epidemics in India commenced in the Deccan in 1949 (Patel *et al* [27]), spreading over all of India in the 1950s, and probably into the adjoin-

TABLE 1. New records since 1940 of rust (*Puccinia* spp.) attacking commercial sugar cane in Asia, Africa, Australia and the Americas

Year reported	Country	Reference
1941	South Africa*	R. Bailey, p.c.
1947	South Africa	McMartin[25]
1949	India	Patel *et al*[27]
1953	USA (Mississippi)*	
	Guatemala*	Baker *et al*[7]
1956	India (Nepal border)	Vasudeva[30]
	Mozambique*	Martin[23]
	Malagasy R.*	do.
	Cuba*	do.
1962	Malagasy R.*	Hughes[19]
1964	Tanzania	Watson[31]
	Rhodesia	Anon[1]
	Mauritius*	Antoine & Perombelon[5]
1965	Reunion*	Hoarau[17]
1967	Mexico*	McGuire & Crandall[24]
1968	Nepal	Khadka & Shah[20]
pre-1969	Uganda, Kenya, Malawi	Bock[10]
1969	Cameroon*	Barat[8]
pre-1970	Zambia	Logan[22] Anon[3]
pre-1975	South Africa	Bailey[6]
1975	Japan	Ohtsu[26]
1977	Taiwan	Hsieh *et al*[18]
1978	Dominican R.*	Presley *et al*[28]
	Australia	Egan & Ryan[15]
	Jamaica	Koike *et al*[21]
	Puerto Rico	
1978-1979	Belize, Cuba, Guadeloupe, Honduras, Mexico, Panama, USA	personal communications

* *identified initially as P. kuehnii*

TABLE 2. Suggested chronology for *P. melanocephala* based on current knowledge and deductions

Year	Country
pre-1941	South Africa
pre-1949	India
1940s or 1950s	Mozambique, Malagasy R.
mid to late 1950s	Nepal
early mid-1960s	Tanzania, Kenya, Uganda, Rhodesia, Malawi, Zambia
1964	Mauritius, Reunion
late 1960s	Cameroon
1975	Japan
1977	Taiwan
1978	Caribbean, Central America, Australia
1979	North America

ing countries. The first African outbreaks were found in 1964 in Tanzania and Rhodesia (Watson[31], Anon[1]). Other East African countries followed within a short period. These were possibly due to spore dispersal from India. In 1975, the pathogen in South Africa was identified as *P. melanocephala* (Bailey[6], Anon[4]).

Further spread occurred eastward with the finding of rust in Japan, Taiwan and Australia in 1975, 1977 and 1978, respectively. Widespread Caribbean and American outbreaks occurred in 1978 and 1979. The initial finding in Dominican Republic was identified briefly as *P. kuehnii.*

Basis for amending previous records

Much circumstantial evidences, and an increasing amount of concrete evidence, showed that the recent records of rust disease contain many inaccuracies. By using varietal susceptibility patterns, Egan[14] concluded that all of the African outbreaks after 1960, the recent American and Australian outbreaks, and the Indian outbreak, were caused by *P. melanocephala*. Several widely distributed varieties, e.g. B4362, Co475, H49-5, Q90, showed high susceptibility in these outbreaks. They were known to be quite susceptible to *P. melanocephala*, but not to *P. kuehnii*.

The identity of the pathogen is being rechecked in several countries. There was not a good understanding of the differences between the two *Puccinia* spp until recently. Understandably, mis-identifications were made in a number of cases.

The pathogen in South Africa was identified in 1975 as being *P. melanocephala* (Anon[4]). This was true for all varieties, even for Co301 which has continued to show the same type of rust symptoms throughout the period from 1941 to 179 (Bailey, p.c.). *P. kuehnii* was never detected. Therefore, it is virtually certain that the rust disease recorded in 1941 in South Africa was also caused by *P. melanocephala*. This alters the whole concept of the spread of this disease in Africa.

Rust disease specimens from Cameroon and Reunion have been compared with specimens from the 1978 outbreak on Guadaloupe at the IRAT laboratories in Paris. Baudin (p.c.) indicated that the same pathogen, *P. melanocephala*, was responsible in all three cases.

A proposed chronology for P. melanocephala spread

All the available evidence indicated that *P. melanocephala* is more widespread thus, a more important pathogen that was realized. Taking into account the rapid spread demonstrated by the pathogen in the Caribbean and Australia, and using all available information on the disease, the past records have been amended or re-interpreted to produce what is believed to be a more accurate record. The suggested chronology is given in Table 2.

Asia

The fungus was identified separately in 1907 and 1944 in India, on large grasses related to sugar cane. In view of its subsequent history, it is likely that it also occurred on sugar cane from time to time. Chona and Munjal[11], in discussing the first Indian rust epidemic on commercial cane in the Deccan in 1949, noted that specimens of a rust had been collected from (commercial) can in 1919 and 1926.

By 1956, rust was present throughout India. Vasudeva[30] reported it on cane near the Nepal border. The first record located for Nepal was in 1968 (Khadka and Shah[20]). There were no other known records for adjoining countries, which seems unlikely

P. melanocephala was identified in Japan in 1975 and in Taiwan in 1977. These findings probably indicated further spread of the pathogen, although it would be necessary to check the old records of *P. kuehnii* for these two countries.

Africa

P. melanocephala rust in Africa can be dated back to at least 1941, and the outbreak was possibly due to an accidental introduction from India into South Africa. The heavy rusting in Co301 in South Africa in the 1940s and early 1950s, when it was a major variety, certainly would have produced enough wind-borne inoculum to infect other cane-growing areas in southern Africa.

Mozambique and Malagasy Republic probably acquired rust in the mid-1940s to early 1950s. Spread then continued into other countries in southern and eastern Africa, up to Kenya in the early to mid-1960s, and across to Mauritius and Reunion in 1964. In the late 1960s, spread continued westward into Angolo where B4362 was heavily rusted by 1970 (Bernard[9]), and into Cameroon by 1969.

There have been no reports of rust in any other countries in West Africa.

Australia and the Americas

The first records of *P. melanocephala* in these geographical zones were in 1978. The evidence indicated strongly that these were very recent introductions, possibly due to wind-borne spread from an as yet unidentified source (Egan and Ryan[16], Bernard[9]). Wind-borne dispersal of spores, from the earliest outbreaks in the Caribbean, has resulted in rust disease outbreaks in other islands, North America and Central America. At the time of writing (late May 1979), the disease has not been recorded in South America.

CONCLUSION

World distribution

The ISSCT World List of diseases and their distribution (Egan[13]) was taken

as the basis for the following recommended alterations.

It would be necessary to recheck the identity of the sugar cane rust pathogen(s) in all countries, unless this was done recently. Australian specimens of both *P. melanocephala* and *P. kuehnii* were sent to the Commonwealth Mycological Institute in 1978, and were confirmed correct by Dr. J.E.M. Mordue.

Puccinia melanocephala

No deletions are proposed. As of May 1979, 19 additional countries should be added, viz., Angola, Australia, Belize, Cameroon, Cuba, Dominican Republic, Guadeloupe, Honduras, Jamaica, Japan, Mauritius, Mexico, Mozambique, Nepal, Pamama, Puerto Rico, Taiwan, U.S.A. (Florida) and Zambia. Some of the very recent American additions are not supported by published references at the time of writing, but should be in due course. It is almost certain that many more countries in the Americas will be added over the next few years. The full list of 29 countries is shown in Table 3.

TABLE 3. The rust diseases of sugar cane and their distribution.

Puccinia melanocephala H. & P. Syd.				
Angola	Dominican R.	Kenya	Nepal	Tanzania
Australia	Guadeloupe	Malagasy R.	Panama	Uganda
Belize	Honduras	Malawi	Puerto Rico	U.S.A.
Cameroon	India	Mauritius	Reunion	Zambia
China	Jamaica	Mexico	South Africa	Zimbabwe
Cuba	Japan	Mozambique	Taiwan	
Puccinia kuehnii	Butl.			
Australia	Fiji	Japan	Pakistan	Taiwan
Burma	India	Malaysia	Philippines	Thailand
China	Indo-China	New Caledonia	Samoa	
Guam	Indonesia	New Guinea	Sri Lanka	

Puccinia kuehnii

Cameroon, Malagasy Republic, Mauritius, Mozambique, Reunion, South Africa are proposed to be removed from the list. Three of these were shown to be mistaken identifications in the past. The same is true with the other three when they are re-examined. Three additions are proposed. They are Guam, Malaysia, and New Caledonia. These are supported by a specimen (Malaysia) or adequate references (Anon[3]). No action is proposed on:

(i) the reported presence in the Americas (this matter needs to be investigated further),

(ii) the reference to Egypt (Anon[2], Egan[12]) since this is not supported elsewhere.

The full list of 18 countries is shown in Table 3. It is probable that *P. kuehnii* has never been present in Africa and the adjacent islands. Unless the few American reports can be confirmed, as discussed previously, *P. kuehnii* seems to be confined to the Asian-Australian-Pacific region.

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The full list of 18 countries is shown in Table 3. It is probable that *P. kuehnii* has never been present in Africa and the adjacent islands. Unless the few American reports can be confirmed, as discussed previously, *P. kuehnii* seems to be confined to the Asian-Australian-Pacific region.

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UNA REVISION DE LA DISTRIBUCION MUNDIAL DE LAS ESPECIES DE *PUCCINIA* QUE ATACAN LA CAÑA DE AZUCAR

B. T. Egan

RESUMEN

Se revisaron los informes redactados a partir de 1940 sobre espécies de *Puccinia* que atacan la caña de azúcar. En varios casos, el patogeno habïa sido identificado inicialmente como *P. kuehnii,* pero en realidad era *P. melanocephala.* La identidad del patógeno debe ser reexaminada en todos los païses a menos que la identificación se haya hecho recientemente.

P. kuehnii probablemente ocurre solamente en la región Asiática-Australiana del Pacifico, a menos que se confirmen los informes Americanos. Todos los informes Africanos anteriores parecen ser erróneous.

P. melanocephala tiene una distribución mucho más amplia y es probable que pueda ser identificado en muchos païses en los próximos años. Se surgirió una cronologïa modificada para la distribución de este patogeno. Se han presentado listas de distribución mundial para ambos patógenos.

CONSIDERATIONS OF APPEARANCE OF SUGARCANE RUST DISEASE IN THE DOMINICAN REPUBLIC

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ABSTRACT

Sugar cane rust disease incited by the fungus *Puccinia melacocephala* (H. and P. Sydow) was detected at the beginning of July 1978 on variety B 43-62, which constitutes the first report of the Dominican Republic as well as in the Western Hemisphere. Although the rust has been reported in other countries of this area, the etiology of these outbreaks do not correspond to the same pathogen, but to *Puccinia kuehnii* (Kr.) Butl. The rust outbreak in this area is expected when clouds were present and when rainfall and temperature were favorable for its development.

In the Dominican sugarcane industry the losses due to rust are appreciable. The disease is being controlled by planting resistant varieties.

INTRODUCTION

Sugarcane rust disease incited by the fungus *Puccinia melanocephala* (H. and P. Sydow) was observed for the first time in the Dominican Republic on July 3, 1978.

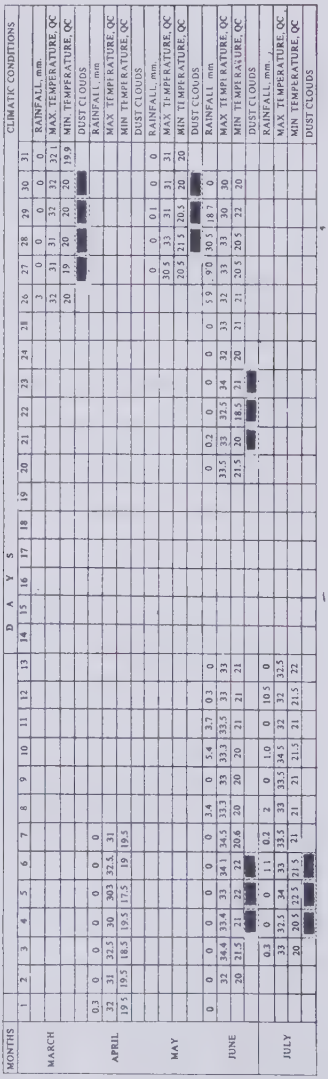
The rust was first identified by F. Bernard *et al.* on July 6, 1978 as *Puccinia kuehnii* (Kr) Butl., but based on teliospore morphology, the causal agent of the rust on sugarcane in the Dominican Republic was later identified by Flora Pollack[1] as *Puccinia melanocephala* H. and P. Sydow.

This disease and the pathogen have never been found in this country before. Many persons have mistaken rust for other foliar diseases as "Ring Spot" (*Leptosphaeria sacchari* VAN BREDA DE HAAN) and some nutritional deficiencies such as those that occurred before in other countries.

The sugarcane variety B 43-62, which is highly susceptible to rust in the Dominican Republic, has been grown for over 20 years here but the symptoms of

rust were never observed. The organism which was reported to be causing rust in Cuba seems to be different from the organism observed here in the Dominican Republic because of its aggressiveness and rapid dissemination. The disease has been observed in all of the sugarcane fields where the variety B 43-62 was grown. The behavior and the morphological characteristics of the causal organism has made investigators to think that this is the first report of this type of rust in the Western Hemisphere (6, 7).

TABLE 1. Climatic data and predominant atmospheric phenomenons before the appearance of outbreaks of "rust" (*P. melanocephala* H. and P. Sydow) in the region of "Los Llanos", where the rust was detected for the first time in Dominican Republic in 1978.



MONTHS	DAYS																													CLIMATIC CONDITIONS
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
MARCH																										3	0	0	0	0	0	RAINFALL, mm.
																										32	31	31	32	32	32.1	MAX. TEMPERATURE, QC
																										20	19	20	20	20	19.9	MIN. TEMPERATURE, QC
																											[redacted]					DUST CLOUDS
APRIL	0.3	0	0	0	0	0	0																									RAINFALL, mm.
	32	31	32.5	30	30.3	32.5	31																									MAX. TEMPERATURE, QC
	19.5	19.5	18.5	19.5	17.5	19	19.5																									MIN. TEMPERATURE, QC
																																DUST CLOUDS
MAY																											0	0	0.1	0	0	RAINFALL, mm.
																											30.5	33	31	31	31	MAX. TEMPERATURE, QC
																											20.5	21.5	20.5	20	20	MIN. TEMPERATURE, QC
																																DUST CLOUDS
JUNE	0	0	0	0	0	0	0	3.4	0	5.4	3.7	0.3	0							0	0.2	0	0	0	0	5.9	9.0	30.5	18.7	0		RAINFALL mm
	32	34.4	33.4	33	34.1	34.5	33.3	33	33.3	33.5	33.5	33	33							33.5	33	37.5	34	32	32	32	33	33	30			MAX. TEMPERATURE, QC
	20	21.5	21	22	22	20.6	20	20	20	20	21	21	21							21.5	20	18.5	21	20	21	21	20.5	20.5	22	20		MIN. TEMPERATURE, QC
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]								[redacted]	[redacted]	[redacted]	[redacted]								DUST CLOUDS
JULY			0.3	0	0	1.1	0.2	2	0	1.0	0	10.5	0																			RAINFALL, mm.
		33	32.5	34	33	33.5	33	33.5	34.5	32	32	32.5																			MAX. TEMPERATURE, QC
		20	20.5	22.5	21.5	21	21	21	21.5	21	21.5	22																			MIN. TEMPERATURE, QC
			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]																			DUST CLOUDS

ORIGIN

How could rust be carried to the Caribbean? By a series of atmospheric and climatic phenomenons, we speculate that the causal organism of rust was carried through dust clouds which settled in this area (3, 4). The fungus was able to establish itself in the presence of favorable weather condition (Table 1) and susceptible host.

DISCUSSION

B 43-62 (the best sugarcane variety in the Dominican Sugarcane Industry) occupies 25.28% of the total acreage in the Dominican Republic. The presence of favorable climatic conditions enabled the fungus to multiply very rapidly resulting in devastation of our sugar industry.

The damages caused by this disease are higher when cane plants are attacked at 3-4 months old. The negative effects contribute to ratoons of 18 months instead of regular ratoons of 12 months. Thus, the three harvesting of sugarcane obtained from regular ratoon and reduced to two harvesting when rust is present. The experimental loss is 33.3% tons in a 3-year cycle.

The more satisfactory and economic control of rust is replacing susceptible varieties with resistant ones, if possible, of high yields and resistant to other diseases as "smut" (*Ustilago scitaminea* Sydow). The reaction of different varieties available in "Duquess Experimental Station" are shown in Table 2. The relative humidity, temperature and rainfall during the two month period are shown in Table 3.

TABLE 2. Reactions of 42 sugarcane varieties after 2 months exposure to "rust" under field conditions.

REACTIONS				
Highly resistant	Resistant	Average	Susceptible	Highly Susceptible
PR–980		PR–65625		B–4362
PR–641791	PR-1242	Co–853	H–50-775	PR–62285
PR–65325	PR-61902	PR–62258	H–50-775	PR–61632
PR–11-66	RD-7502	Co–421	RD–7409	PR–63489
PR–62195	RD-7501	RD–7519	RD–7515	RD–7506
PR–11-48	PINDAR	PR–641548	RD–7518	
PR–62739	PR-6266	PR–1152	RD–7516	
PR–1249	PR-641939	RD–7511		
	CB-44-105	B–47-310		
	PR-1002	PR–64223		
	CR-6101	PR–63488		
	RD-7505			
	UCW-5465			

TABLE 3. Relative humidity, temperature and rainfall during the experiment

Month	Temperature °C Max.	Min.	Rainfall mm.	Humidity %
Dec. 1978	30.6	16.5	75.2	73%
Jan. 1979	30.2	15.7	15.8	71%
Feb. 1979	31.4	15.1	0.8 (day 11)	70%

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CONSIDERACIONES SOBRE LA INCIDENCIA DE LA ROYA DE LA CAÑA DE AZUCAR EN LA REPUBLICA DOMINICANA

F. A. Bernard

RESUMEN

Se encontro por primera vez tanto en la Republica Dominicana como en el Hemisferio Occidental la roya de la caña de azucar causada por el hongo *Puccinia melanocephala* (H. & P. Sydow) a principios del mes de julio de 1978 en la variedad B 4362. Aunque se ha reportado la roya en otros paises, la misma se ha identificado como *Puccinia kuehnii* no como *P. melanocephala.* Se especulo que el origen de la roya en la

Republica es de Africa por la presencia de nubes cargadas de polvo. La temperatura y la lluvia son favorables al desarrollo de la enfermedad.

Las perdidas causadas por la roya en la Republica son apreciables. Se empezaron a desarrollar metodos para controlar la enfermedad la mediante la siembra de las variedades resistantes.

EVALUATION OF SUGARCANE FOR RESISTANCE TO RUST IN PUERTO RICO AND IN THE DOMINICAN REPUBLIC

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ABSTRACT

Forty-one local and commercial varieties of sugarcane and promising selections were evaluated for reactions to infection by *Puccinia erianthi* after artificial inoculations in Puerto Rico. B 4362, Co 798, Q 90, PR 1124, PR 64-2705, PR 63-1165, PR 67-3129, and PR 70-3413 were found highly susceptible to rust while PR 980, PR 1059, PR 1141, PR 61-632, PR 62-258, PR 62-739, PR 63-488, PR 63-525, PR 64-1548, PR 65-625, PR 65-2638, PR 67-245, PR 67-1070, PR 68-2319, and PR 69-2030, were found to be highly resistant. Of the three methods used for artificial inoculations, wraping the young shoot with rust affected leaves seems to be the most effective. Thirty-four varieties of sugarcane were evaluated for reactions to infection by *Puccinia erianthi* after artificial inoculation in the Dominician Republic. B 4362, B 74-224, B 74-355, CB 44-105, PR 1048, PR 1050, PR 1250, and PR 61-632 were found highly susceptible to the rust while B 52313, PR 1148, and PRO 980 were found highly resistant.

INTRODUCTION

Sugarcane rust caused by *Puccinia erianthi* Padw. & Khan, is a leaf disease of great importance in India, Australia (3) as well as in South Africa *(Bailey*[1]) *Rust is widely distributed in the sugarcane-growing countries of the Eastern Hemis-* Rust is widely distributed in the sugarcane-growing countries of the Eastern Hemisphere. The disease has also been reported recently in the Dominican Republic (Bernard[2], Presley *et al*[5] and Whittle[7]), Jamaica and Florida. It occurs mostly under humid conditions and warm climate.

In Puerto Rico, rust was reported for the first time on sugarcane seedlings 78-57 at the Fortuna Substation along the southern coast of the island by Liu[4] in 1978. It was subsequently found in Lajas, Isabela, Gurabo, Humacao and Yabucoa areas on thousands of seedlings used in the sugarcane breeding program. Commercial varieties PR 1124, Co. 798, and Q 90 were also affected by the rust. The

indirect loss caused although difficult to assess, can reach considerable proportion. Because some of the local commercial varieties and promising selections lack resistance to rust, a search for sources of resistance in the sugarcane breeding materials was initiated. This report describes reactions of some selections and local commercial varieties to inoculations with *P. erianthi.*

MATERIALS AND METHODS

Thirty-three varieties of sugarcane in the Dominican Republic (Table 1) and 41 varieties and promising selections (Table 2) from the breeding program in Puerto Rico, were evaluated for reactions to *P. erianthi* after artificial inoculations. In Puerto Rico three single-eyed seedpieces of each variety were planted on February 8, 1979 in 21, 12 inches plastics bags. The bags were placed on the benches under a 75% saran cloth. In the Dominician Republic, 5 double-eyed seedpieces were planted in a metal flat. The flats were kept in the greenhouse. Four weeks after germination, the plants were inoculated by 1) spraying with a suspension of urediospores (approximately 200 spores per ml), in both locations. Two treatments i.e., 1) dusting with urediospores, and 2) wrapping the young shoot with a piece of rust affected leaf were added to the experiments conducted in Puerto Rico. In all cases, inoculated plants were covered with polyethylene bags overnight. The relative air humidity during the inoculation period was 65-70% and the average daily temperature fluctuated from 75° to 85°F.

In Puerto Rico, disease severity and lesion type in each variety were recorded at 14 days and at 30 days after inoculation. The following modified rust infection types described by Stakmen and Harrar[6] were used to determine varietal reaction to *P. erianthi:*

Infection types

1 Immune – no rust pustules developed, but small flecks of dead host tissue sometimes are present.

2-3 Very resistant – rust pustules extremely small and surrounded by dead areas. Approximately 10-15% of the total leaf area was affected.

4-5 Moderately resistant – pustules small to medium; usually in green islands of host tissue surrounded by a band of chlorotic or dead tissue. Approximately 20-25% of the total leaf areas was affected.

6-7 Moderately susceptible – pustules medium in size, usually separate, no dead areas, but chlorotic area may be present, especially under favorable conditions. Approximately 30-35% of the total leaf areas was affected.

8-9 Very susceptible – pustules large and often united, no dead tissue, but there may be leaf yellowing under unfavorable growing conditions.

In the Dominican Republic, disease severity and lesion types of each variety were recorded at 30 days after inoculation. B 4362, a highly susceptible variety

and PR 980, a highly resistant variety, were included as controls. Based on severity and lesion types produced, the above-mentioned varieties were rated in comparison with PR 980 and B 4362.

RESULTS AND DISCUSSIONS

In Puerto Rico, reactions to *P. erianthi* were grouped according to infection types and the percentage of diseased leaf area at each rating time (Table 1). Varieties B 4362, Co 798, Q 90, PR 1124, PR 64-2705, PR 63-1165, PR 67-3129, and

TABLE 1. Reaction of some sugarcane varieties to *Puccinia erianthi* in the dominican Republic.

Variety	Reaction	Variety	Reaction
B 52-313	HR	B 42231	S[1/]
PR 1148	HR	B 51-129	S
PR 980	HR	B 70-171	S
CP 52-43	R	CO 853	S
Pindar	R	Q 90	S
PR 1028	R	PR 61-902	S
PR 1248	R	PR 65-556	S
UCW 54/65	R	B 4362	HS
B 49119	MR	B 74-224	HS
B 74-150	MR	B 74-355	HS
BR 67-08	MR	CB 44-105	HS
CR 67-400	MR	PR 1048	HS
PR 1002	MR	PR 1050	HS
PR 1141	MR	PR 1152	HS
PR 65-625	MR	PR 1250	HS
RD 74-09	MR	PR 61-632	HS
RD 74-10	MR		

1/ HR = Highly resistant
R = Resistant
MR = Moderately resistant
S = Susceptible
HS = Highly susceptible

PR 70-3413 showed large and often united pustules and no dead tissues. Therefore, they were rated as highly susceptible to the rust. On the other hand, PR 980, PR 1059, PR 1141, PR 61-632, PR 62-258, PR 62-739, PR 63-488, PR 63-525, PR 64-1548, PR 65-625, PR 65-2638, PR 67-245, PR 67-1070, PR 68-2319, and PR 69-2030 did not form rust pustule after inoculation. Only small flecks of dead tissues occurred on the leaves. Accordingly, they were rated as highly resistant or immune. PR 1148, PR 68-2246, PR 69-3065 showed medium size pustules which were usually separate and no dead tissues. However, clorotic areas may be present especially under favorable conditions. They were, therefore, rated as moderately susceptible. In the Dominican Republic, reactions to *Puccinia erianthi* were rated according to infection types and severity of the disease. Varieties B-4362, B 74-224, B 74-355, CB 44-105, PR 1048, PR 1050, PR 1250, and PR 61-632 showed large and often united pustules and no dead tissues and were rated as highly susceptible

to the rust. On the other hand, PR 980, B 52-313, and PR 1148 showed no developed rust pustules but small flecks of dead host tissues and were rated as highly resistant (Table 2).

TABLE 2. Reaction of some sugarcane varieties to *Puccinia erianthi* in Puerto Rico

Variety	Infection types					
	Spore Suspension		Dusting		Wrapping	
	14 days	30 days	14 days	30 days	14 days	30 days
B 34-104	1	1	1	1	1	1[a]
B 42231	1	2	1	2	1	2
B 4362	8	9	8	9	9	9
B 45-733	1	1	1	1	1	1
B 47-310	1	1	1	1	1	1
CB 44-105	1	1	1	1	1	1
Co 798	8	9	8	9	9	9
CP 52-43	4	5	4	5	5	5
H 32-8560	1	1	1	1	1	1
Q 90	8	9	8	9	9	9
PR 980	1	1	1	1	1	1
PR 1013	2	2	2	2	2	3
PR 1028	2	3	2	3	3	3
PR 1059	1	1	1	1	1	1
PR 1141	1	1	1	1	1	1
PR 1124	8	9	8	9	9	9
PR 1148	6	7	6	7	7	7
PR 61-632	1	1	1	1	1	1
PR 62-258	1	1	1	1	1	1
PR 62-739	1	1	1	1	1	1
PR 62-469	1	2	1	2	2	2
PR 63-227	1	2	1	2	2	2
PR 63-1165	9	9	8	9	9	9
PR 63-488	1	1	1	1	1	1
PR 63-525	1	1	1	1	1	1
PR 64-1548	1	1	1	1	1	1
PR 64-2705	8	9	8	9	9	9
PR 65-1128	2	3	2	3	3	3
PR 65-199	2	3	3	3	3	3
PR 65-625	1	1	1	1	1	1
PR 65-2638	1	1	1	1	1	1
PR 66-1240	3	3	3	3	3	3
PR 67-245	1	1	1	1	1	1
PR 67-1070	1	1	1	1	1	1
PR 67-3129	9	9	9	9	9	9
PR 68-2246	5	6	5	6	6	6
PR 68-2319	1	1	1	1	1	1
PR 69-222	5	6	6	6	6	6
PR 69-2030	1	1	1	1	1	1
PR 69-3065	6	7	6	7	7	7
PR 70-3413	8	9	9	9	9	9

[a] Each value is the mean of 7 replications

The results obtained from both localities were in consonance with each other (Bernard[2]). However, B 42231 and PR 61-632 which were highly susceptible in the Dominican Republic, were found highly resistant in Puerto Rico. On the other hand, PR 1148 which was highly resistant in the Dominican Republic was found

moderately susceptible in Puerto Rico. This indicates that physiologic races might have occurred in these two countries.

In Puerto Rico, the results obtained in the artificial inoculations agree with the observations by Mr. T.L. Chu and co-workers under natural field infection. Varieties B 4362, Co 798, Q 90, PR 1124, PR 67-3129, and PR 70-3413 which were highly susceptible in artificial inoculations were also found highly susceptible in commercial fields under natural infection.

Of the three methods used for artificial inoculations, wrapping the young, shoot with rust affected leaves seems to be the most effective.

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LA EVALUACION DE LA CAÑA DE AZUCAR PARA LA RESISTENCIA DE LA ROYA EN PUERTO RICO y LA REPUBLICA DOMINICANA

Lii-Jang Liu y F. Bernard

RESUMEN

Se evaluaron 40 variedades locales y comerciales de la caña de

azucar y selecciones prometedoras para las reacciones a la infeccion por *Puccinia erianthi* despues de inoculaciones artificiales. B 4362, Co 798, Q 90, PR 1124, PR 63,227, PR 63-1165, PR 67-3129 y PR 70-3413 fueron encontradas altamente susceptibles a la roya mientras PR 980, PR 1059, PR 1141, PR 61-632, PR 62-258, PR 62-739, PR 63488, PR 63-525, PR 64-1548, PR 65-626, PR 62-2638, PR 67-245, PR 67-1070, PR 68-2319 y PR 69-2030 fueron encontradas altamente resistentes. Entre los tres metodos usados para la inoculacion artificial envolver el cogollo joven con la hojas afectadas por la roya parecio ser el metodo de inoculacion mas efectivo. Se hizo la evaluacion de treinta y cuatro variedades de caña de azucar sobre los reacciones a la infeccion del hongo *Puccinia erianthi* despues de su inoculacion artificial en la Republica Dominicana. Los variedades B 4360, B 74-224, B 74-355, CB 44-105, PR 1048, PR 1050, PR 1250 y PR 61-632 fueron encontrados altamente susceptibles, mientras que los B 52313, PR 1148 y PR 980 fueron altamente resistentes.

RUST OF SUGARCANE IN THE DOMINICAN REPUBLIC

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ABSTRACT

Thirty-four varieties of sugarcane were evaluated for reactions to infection by *Puccinia erianthi* after artificial inoculation. Varieties B 4362, B 74-224, B 74-355, CB 44-105, PR 1048, PR 1050, PR 1250, and PR 61-632 were found highly susceptible to the rust while B 52-313, PR 1148, and PR 980 were highly resistant. Eight fungicides, Plantvax, Calixin-M, Poliran-Combi, Fermate, Vitigran Verde, Daconil 2787, Dithane M-45, and Bavistin were tested in the fields for their relative effectiveness in the control of the rust. Among the fungicides tested, Plantvax seemed to be the most effective for inhibiting pustul formation.

INTRODUCTION

Among the fungal diseases affecting leaves of sugarcane, rust caused by *Puccinia erianthi* Padw. and Khan[2], is probably the most important. Rust was observed on July 3, 1978 by Mr. Raul Hodge at San Felipe farm in the Vicini holdings[5]. The fungus was identified tentatively on July 7, 1978 in the Phytopathology Laboratory of the Duquesa Experiment Station as *Puccinia kuehnii* (Kr.) Butl.[1] Recent surveys made by Liu and Bernard[4] in various ecological regions of the sugarcane plantation owned by the Consejo Estatal del Azucar (CEA) in the Dominican Republic, showed the presence of uredial pustules the disease in Central Ozama, Central Boca Chica, Central Barahona, and Central Montellano and the rust was identified as *P. erianthi.* The disease has caused concern since several commercial varieties including B 4362, a major commercial variety occupying more than 31% of the total cane area at CEA, have been affected.

The present study was undertaken to know the reaction of some cane varieties to rust and to determine the effects of various fungicides on rust pustul formation.

MATERIALS AND METHODS

Thirty-four varieties of sugarcane, B 52-313, PR 1148, PR 980, CP 52-43, Pindar, PR 1028, PR 1248, UCW 54/65, B 49119, B 74-150, BR 67-08, CR 67-

400, PR 1002, PR 1141, PR 65-625, RD 65-625, RD 74-09, RD 74-10, B 42231, B 51-129, B 70-171, Co 853, Q 90, PR 61-902, PR 65-556, B 4362, B 74-224, B 74-355, CB 44-105, PR 1048, PR 1050, PR 1152, PR 1250, and PR 61-632 were evaluated for reactions to *P. erianthi.* Five double-eyed seedpieces of each of the above-mentioned varieties were planted in a metal flat. The flats were placed on the benches in a greenhouse. Four weeks after germination, plants were inoculated by spraying with urediospores suspension. The relative air humidity during the inoculation period was between 65-75% and the average daily temperature fluctuated from 75° to 85°F. Disease severity and lesion types produced on each variety were recorded at 30 days after inoculation. Highly susceptible variety (B 4362) and highly resistant variety (PR 980) were included as controls. Based on severity and lesion types produced, the above-mentioned varieties were rated as compared with those produced by PR 980 and B 4362.

In the study of the effect of various fungicides on symptom expression of *P. erianthi,* two month old canes of B 4362 were sprayed with the fungicides, Plantvax, Calixin-M, Poliran-Combi, Fermate, Vitigran Verde, Daconil 2787, Dithane M-45, and Bavistin, each at the dosages recommended by their manufacturers (table 2). The numbers of chlorotic lesions as well as uredial pustules per 2 cm^2 were recorded periodically.

TABLE 1. Reaction of some sugarcane varieties to *Puccinia erianthi* in the Dominican Republic.

Variety	Reaction[1]	Variety	Reaction[1]
B 52-313	HR	B 42231	S
PR 1148	HR	B 51-129	S
PR 980	HR	B 70-171	S
CP 52-43	R	CO 853	S
Pindar	R	Q 90	S
PR 1028	R	PR 61-902	S
PR 1248	R	PR 65-556	S
UCW 54/65	R	B 4362	HS
B 49119	MR	B 74-224	HS
B 74-150	MR	B 74-355	HS
BR 67-08	MR	CB 44-105	HS
CR 67-400	MR	PR 1048	HS
PR 1002	MR	PR 1050	HS
PR 1141	MR	PR 1152	HS
PR 65-625	MR	PR 1250	HS
RD 74-09	MR	PR 61-632	HS
RD 74-10	MR		

[1] *HR = Highly resistant* *S = Susceptible*
R = Resistant *HS = Highly susceptible*
MR = Moderately resistant

RESULTS AND DISCUSSION

Based on infection types and severity of the disease, varieties B 4362, B 74-224, B 74-355, CB 44-105, PR 1048, PR 1050, PR 1250, and PR 61-632 were rated as highly susceptible to the rust, whereas PR 980, B 52-313, and PR 1148 were rated as highly resistant (Table 1).

The numbers of chlorotic lesions as well as brown nectrotic pustules per 2 cm^2 leaf area for each fungicides were recorded periodically. The results obtained seem to indicate that Plantvax is the most effective in inhibiting rust formation (Table 2).

TABLE 2. Effect of various fungicides in pustul formation on leaves of sugarcane variety B 4362 by *Puccinia erianthi*

Fungicide	Concentration (9 m/5 gal)	Number of chlorotic lesion per 2 cm^2 leaf area	Number of brown necrotic pustules per 2 cm^2 leaf area
Plantvax	35	34 abc[1]	34 a[1]
Calixin-M	120	21 a	71 a
Poliran-Combi	34	43 bc	50 a
Fermate	34	51 c	45 a
Vitigran Verde	76	36 abc	73 a
Daconil 2787	34	23 a	51 a
Dithane M-45	36	41 bc	84 a
Bavistin	14	29 ab	70 a
Control	—	36 abc	103 a

[1] *Averages followed by the same letter did not differ significantly at 5% DMRT.*

The results obtained in the study of reactions of sugarcane varieties to rust agree mostly with the findings by Liu[3] who reported that variety PR 980 was highly resistant in Puerto Rico. However, PR 61-632 which was resistant in Puerto Rico was found highly susceptible in the Dominican Republic.

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LA ROYA DE LA CAÑA DE AZUCAR EN LA REPUBLICA DOMINICANA

Filiberto M. Bernard y Lii-Jang Liu

RESUMEN

Se hizo la evaluacion de treinta y cuatro variedades de caña de azucar sobre las reacciones a la infeccion del hongo *Puccinia erianthi* despues de su inoculacion artificial. Las variedades B 43-62, B 74-224, B 74-355, CB 44-105, P R 1048, P R 1050, P R 1250 y P R 61-632 fueron encontradas altamente susceptibles, mientras que las B 52-313, P R 1148 y P R 980 fueron altamente resistentes. Se probo en el campo la efectividad relativa en el control de la roya de los fungicidas Plantvax, Calixin-M, Poliran-Combi, Fermate, Vitigran Verde, Daconil 2787, Dithanes M-45 y Bavistin. Entre los fungicidas probados, el Plantvax parecio ser el mas efectivo en la inhibicion de la formacion de las pustulas.

SELECTION OF SUGAR CANE RESISTANT TO RED ROT

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ABSTRACT

The study was made to determine the resistance of some varieties, clones and hybrids of sugar cane to red rot caused by *Colletotrichum falcatum* Went, with offspring of high resistance to the disease. The canes tested showed varying degrees of resistance to the disease.

INTRODUCTION

Sugar cane red rot is wide-spread in different degrees of incidence in most cane growing countries but the degree of severity varies. Resistance to the disease is one of the criteria used in selecting varieties of sugar cane to be planted. Red rot is severe in Brazil, U.S.A., Philippines and several other countries.

It is an endemic disease in the subtropical areas where very often, new strains of the fungus emerge (Srinivasan[8]). India for example, is divided according to Srinivasan in three main regions: 1) the tropical areas where susceptible varieties may be cultivated; b) subtropical areas where with certain precautions, moderately susceptible varieties may be cultivated; c) the subtropical areas where the disease is endemic and only resistant varieties may be cultivated.

The existence of the disease in Cuba has been reported by Jhonston since 1915 (5) but not frequently encountered until the present decade when it occurred in majority of our sugar cane plantations (Alfonso *et al*[1]).

Starting from 1972 the pathogenic fungus as well as the disease have been studied by the Mycology Team from the Sugar Cane Research Institute.

Results of studies by Alfonso[2] and Gonzalez[3] showed that at present there are at least three strains of the fungus in Cuba. Possibly more will be identified later.

Due to the importance of the disease in Cuba, tests of the resistance of some varieties being grown and the clones from the different stages of selection were started.

MATERIALS AND METHODS

Twenty-five, nine-month old plants or clones in the field were inoculated by the Singh and Budhraja[6] method. The fungus isolated from B 4362, a variety susceptible to the disease, was used in inoculation. Three months after inoculation, evaluation of reactions of clones was done according to the following scale (Alfonso[4]):

Degree I – Highly resistant, HR
Degree II – Resistant, R
Degree III – Moderately Resistant, MR
Degree IV – Susceptible, S
Degree V – Very Highly Susceptible, HS

Srinivasan[8] criterion was also considered in establishing the previous degrees. All inoculations were done at the Sugar Cane Experimental Station in Jovellanos, Mantanzas.

RESULTS AND DISCUSSION

Table 1 shows that among the susceptible commercial varieties, only B 4362 was planted in a considerably large acreage in Cuba. This variety had been infected with the disease in all the country's plantation areas. Majority of the varieties on trials however, have shown some degree of resistance (I, II or III) to the disease.

TABLE 1. Commercial varieties and their degree of resistance to sugar cane red rot.

Variety	Degree of resistance	Distribution in the country		
		Widely	Partial	Limited
Ja 60-5	I	x		
My 5514	II		x	
B 42231	I		x	
C 97-59	I			x
PR 980	II		Ix	
C 87-51	II			
My 53174	II			x
My 54129	III		x	
NCo 310	III			IIx
CP 52-43	III		IIx	
My 5715	III		x	
Co 281	IV			Vx
B 4362	IV	Vx		
My 5458	IV			x
Pomex 72	IV			x
My 5489	V			x
My 54130	V			x

Result of artificial inoculation concurred fully with those observed under natural conditions in different areas of the country.

Resistance to sugar cane red rot was observed in most varieties and hybrids. When very resistant progenitors like B 42231 and C 97-59 were used, the degree of resistance was higher.

TABLE 2. Degree of Resistance of some hybrids to sugar cane red rot

Progenition	Total amount Evaluated	Resistance Degree 1	II	III	IV	V
B 42231 x C 97–59	12	2	1	8	1	
CG 127–45 x C 97–59	5		2	2	1	
My 5514 x C97–59	6		2	4		
C1 41142 x C 97-59	4	1	1	2		
CP 44–155 x May 54129	5			5		
My 53174 x C87–51	4				2	2
Co 281 x My 54129	6		1	1	4	
CP 52–43 x C 227–59	5		1	4		
POJ 2878 x CP 52–43	8			5	1	2

Progenies of hybrid "Badila" trials (Table 3) showed similar results with 42 out of 48 samples from the same population, included in the resistant categories.

TABLE 3. Degree of Resistance of Badila hybrids to sugar cane red rot

Degree	Hybrid	%
I	2	4.17
II	14	27.08
III	27	56.26
IV	5	10.42
V	1	2,08

It is extremely interesting to note that resistance to sugar cane red rot was observed in most of the plants under study. In most clones, the disease outbreak is not present in the natural condition. Only 5 out of almost 200 clones exhibited the natural infection in a set from the Hector Molina sugar mill area. On the other

hand, only 11 out of 3000 hybrids of different progenitors showed symptoms of natural infection in Jovellanos. This precludes the possibility of selecting a variety for production in the later stage that is susceptible to the disease.

CONCLUSIONS AND RECOMENDATIONS

The great majority of sugar cane varieties evaluated showed resistant characteristics to red rot. Only variety B 4362, which is widely cultivated, showed susceptibility to the disease. If high disease resistant progenitors are used, resistant offsprings were also obtained.

Knowing the great importance of the disease in subtropical area, only varieties that are resistant must be planted. Highly susceptible varieties should not be grown due to the possibility that new and more aggressive strains of red rot causing organisms will develop.

Varietal resistant trials must not start at early stages of the selection process in order to make the work simpler. Varietal resistance and fungus strain determination studies in Cuba must be pursued more intensively.

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PODREDUMBRE ROJA DE LA CAÑA DE AZUCAR EN EL PROCESO DE LA SELECCION

Roberto Gonzales Hernandez

RESUMEN

Se hicieron estudios sobre la resistencia de algunas variedades, clones y hibridos la enfermedad de la podredumbre roja, causada por *Colletotrichum falcatum* Went. Los resultados indican que muchas descendencias son altamente resistentes a la enfermedad. Se describen los criterios que se usan en el proceso de selecciones de variedades en relacion con los diferentes grados en la resistencia observada.

ECOTAXONOMICAL ANALYSIS OF NEMATODES OF THE SUGAR CANE *SACCHARUM SPP.* HYBRID IN CUBA

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ABSTRACT

Ecotaxonomical analysis of nematodes of sugar cane in Cuba showed the presence of 194 species, belonging to 2 subclasses, 5 orders, 25 families and 76 genera.

Most of these species belong to the orders Tylenchida (70 spp.) and Dorylaimida (71 species) followed by the orders Rhabditida (26), Enoplida (17) and Chromadorida (10).

According to the ecological classification all the recorded species are distributed as follows: Pararhizobiontics with 83 species (43%); Eusaprobiontics, 4 species (2%); Devisaprobiontics, 35 species (18%); Fitohelmints, 72 species, (37%).

INTRODUCTION

In the guidelines for the social and economical development of Cuba in the five-year period (1976-1980), it is foreseen that cane agriculture should supply high levels of cane for the sugar industry. It is important then to study pests and diseases which injure cane in order to have high production.

Together with the pathogens fungi, viruses and bacteria, nematodes play an important role in the occurrence of disease in sugar cane. The Sugar Cane Research Institute of the Academy of Sciences of Cuba performed ecotaxonomical analysis of nematodes in sugar cane from 1967 to 1978. The final results are presented in this paper.

MATERIALS AND METHODS

The study of the population of nematodes of the sugar cane was carried out during 1967-1978 in the whole territory of Cuba. Samples were taken in all the main regions where cane is grown. The sites where the samples were taken and the name of sugar mills are shown in Figure 1.

The areas under study were divided into Western, Central and Eastern regions for analysing the collected material. Data collected from 95 sugar mills

FIGURE 1. Zone in which samples of sugar cane soils and roots have been taken in Cuba

Western Region	Prov. Pinar del Rio	Of sugar cane mill	Name of the sugar cane mills	Number of fielas visited	Samples taken		Total per fielas
					S	R	
		1	Harlem	2	10	10	20
		2	Manuel Sanguily	2	10	10	20
		3	Pablo de la T. Brau	3	15	15	30
		4	Jose Marti	7	35	35	70
Western Region	Habana						
		5	Abraham Lincoln	2	10	10	20
		6	Eduardo Garcia L.	3	15	15	30
		7	Agusto Cesar Sandino	3	15	15	30
		8	Orlando Nodarse	4	20	20	40
		9	Amistad de los Pueblos	3	15	15	30
		10	C. Manuel Fajardo	3	15	15	30
		11	Habana Libre	2	10	10	20
		12	Camilo Cienfuegos	2	10	10	20
		13	Gregorio Arlee M.	2	10	10	20
		14	Pablo Noriega	2	10	10	20
		15	Boris L. Santa Coloma	3	15	15	30
Western Region	Ciudad de la Habana	16	Manuel Martinez P.	3	15	15	30
Central Region	Matanzas	17	Mexico	6	30	30	60
		18	Reynol Garcia	5	25	25	50
		19	Australia	5	25	25	50
		20	Granma	3	15	15	30
		21	Puerto Rico Libre	1	5	5	10
		22	Cuba Libre	5	25	25	50
		23	Jaime Lopez	2	10	10	20
		24	Humberto Alvarez	2	10	10	20
		25	España Republicana	1	5	5	10
		26	Esteban Hernandez	7	35	35	70
		27	Seis de Agosto	5	25	25	50

		28	Jesus Rabi	1	5	5	10
		29	Jose Smith Comas	6	30	30	60
		30	Victoria de Yagujay	4	20	20	40
		31	Juan Avila	6	30	30	60
		32	Julio Reyes Cairo	1	5	5	10
		33	Sergio Gonzalez	3	15	15	30
		34	Horacio Rodriguez	4	20	20	40
Central Region	Villa Clara	35	Heriberto Duquesne	2	10	10	20
		36	Luis Arcos Bergnes	1	5	5	10
		37	Abel Santamaria	2	10	20	20
		38	26 de Julio	2	10	10	20
		39	Emilio Cordova	2	10	10	20
		40	Osvaldo Herrera	2	10	10	20
		41	Quintin Bandera	4	20	20	40
		42	Marcelo Salado	2	10	10	20
		43	Jorge Riquelme	3	15	15	30
		44	Chiquitico Fabregat	2	10	10	20
		45	Panchito G. Toros	2	10	10	20
		46	10 de Octubre	2	10	10	20
		47	Hector Rodriguez	2	10	10	20
		48	Jorge Washington	1	5	5	10
Central Region	Cienfuegos	49	Espartaco	2	10	10	20
		50	14 de Julio	2	10	10	20
		51	1ro de Mayo	2	10	10	20
		52	Elpidio Gomez	1	5	5	10
		53	Ramon Balboa	1	5	5	10
		54	Martha Abreu	1	5	5	10
		55	Pepito Tey	1	5	5	10
Central Region	Sancti Spiritu	56	Meladio Hernandez	2	10	10	20
		57	Simon Bolivar	3	15	15	30
		58	Uruguay	2	10	10	20
Central Region	Ciego Avila	59	Enrique Varona	2	10	10	20

		60	Ecuador	3	15	15	30
		61	Bolivia	2	10	10	20
		62	Patria O Muerte	2	10	10	20
		63	1ro. de Enero	3	15	15	30
Eastern Region	Camagüey	64	Ignacio Agramonte	1	5	5	10
		65	Carlos M. de Cespedes	1	5	5	10
		66	R. Dominicana	4	20	20	40
		67	Brasil	5	25	25	50
		68	Sierra de Cubitas	3	15	15	30
		69	Haiti	2	10	10	20
		70	Alfredo Alvarez	1	5	5	10
		71	Candido Gonzalez	4	20	20	40
		72	Noel Fernandez	5	25	25	50
		73	Panama	4	20	20	40
Eastern Region	Las Tunas	74	Jesus Menendez	2	10	10	20
		75	Antonio Guiteras	3	15	15	30
		76	Peru	3	15	15	30
		77	Argelia Libre	2	10	10	20
Eastern Region	Holguin	78	Loynaz Hecheverria	3	15	15	30
		79	Lopez Peña	4	20	20	40
		80	Nicaragua	2	10	10	20
		81	Cristino Naranjo	2	10	10	20
		82	Antonio Maceo	2	10	10	20
		83	Guatemala	2	10	10	20
		84	Urbano Noris	2	10	10	20
		85	Rafael Freire	2	10	10	20
		86	Fernando de Dios	5	25	25	50
Eastern Region	Granma	87	Frank Pais	2	10	10	20
		88	F. Castro Ceruto	5	25	25	50
		89	Bartolome Maso	3	15	15	30
		90	Arquimides Colina	3	15	15	30
		91	La Demajagua	2	10	10	20
		92	Enidio Diaz	2	10	10	20
Eastern Region	S. Cuba	93	Chile	2	10	10	20
	Guantanamo	94	Argeo Martinez	2	10	10	20
		95	Paraguay	2	10	10	20

visited and 258 fields by region are given in Figure 1. A total of 2,580 samples of the soil and root were taken to analyze the nematode fauna.

The Baermann funnel method was used to isolate nematodes from the samples. The samples were poured on polyethylene, a thin stool was spread and from it three replications of 20 g of soil were taken so with a replication of 10 g from the root.

RESULTS AND DISCUSSION

Nematode Composition

Laboratory analysis of sugar cane soil and root samples showed 194 species of nematodes represented by 16,890 specimens. According to the classification set by N. J. Filipjev[2], M. B. Chitwood[1], T. Goodey[3] and Paramonov[4,5,6], the nematode species involved belong to two sub-classes — the Adenophorea (van Lenstow, 1905), Chitwood, 1950 and the Secernentea (Van Linstow, 1905) Dougherty, 1958; to 5 orders, the Chromadorida Chitwood, 1933; the Enoplida Chitwood, 1933; the Dorylaimida Pearce, 1942; the Rhabditida Chitwood, 1933 and the Tylenchida Thorne, 1949; to 25 families and 76 genera (Appendix 1).

It can be observed that the largest number of species belong to the orders Tylenchida and Dorylaimida with 70 and 71 species, respectively, followed by the Rhabditida (26), Enoplida (17) and Chromadorida (10).

The aforementioned nematode orders are different not only because of the number involved, but also for the important effects that some of the species have on plants. Phytonematologists place their chief interest in nematodes belonging to the order Tylenchida because most of them are plant parasitic.

A majority of endo- and ecto-parasites that cause phytohelminthose disease to the root system of the sugar cane were found among the species of this order. Further analysis however has to be carried out concerning this group of nematodes and the diseases they bring about.

The order Dorylaimida is mainly made up of species that dwell on the rhizosphere of plants and feed on microorganisms and secretions of the roots. The species of the Longidoridae and Trichodoridae families are the only representatives of this order that have reached a highly developed biological system concerning parasitism. In the evolution process, their morphology is so well developed (especially their sucking apparatus) that they can attach plants and feed on the content of root cells.

All of them usually have a long and strong style through which they can easily enter the epidermis of the cells and suck the juice.

The species of the Chromadorida, Enoplida and Rhabditida orders are

spread widely in the rhizosphere of the sugar cane. Those of the first two orders are primitive species of water and soil, living freely in the latter, feeding on microorganisms and root secretions, so they are not greatly dangerous to plants. The species of the third order are representatives of a saprobiontic environment and participate in the rotting processes of the organic residue of plants.

Considering the great number of interrelationships between nematodes and plants. Professor A. A. Paramonov[4] proposed an ecological classification of nematodes. According to this, nematodes similar in their way of living and feeding integrate a specific ecological group. In this way, nematodes widely spread in the rhizopshere and feeding on the microorganisms of this environment and secretions of the root system of plants, compose the Pararhizobiontic group. Nematodes which feed on rotten remains of plants are the Eusaprobiontic and those which are nourished from living and dead tissues are named Devisaprobiontic. The last group of nematodes which feed only on living tissues of the plants are the phytohelmints. At the same time, this group is subdivided in: phytohelmints of specific pathogenic effect, causing a determined type of diseases on plants; and phytohelmints of non-specific pathogenic effect, which nourish themselves with the fungus flora.

By arranging the nematodes, into the four mentioned ecological groups above, it can be seen that the greatest number of species is located in the soil (Table 1). The Pararhizobiontic group has 83; and the Phytohelmint group has 72. The Phytohelmints prevail in the roots (62 species), most of which compose the subgroup of specific pathogenic effect (41), endo and ectoparasites of plants. Secondly is the Pararhizobiontic group (73). The Devisaprobiontic group is in the third place and the number of species of the Eusaprobiontic group is generally less important (4 species).

The nematode fauna then concerning the sugar cane is broad and it is represented by all the ecological groups of nematodes, beginning with those primitive species of water and soil unto the highly-developed parasites of plants, the Phytohelmints.

TABLE 1. Number of species in four ecological groups of nematodes

Ecological groups	Total number of species	
	Soil	Root
Pararhizobiontics	83	73
Eusaprobiontics	4	2
Devisaprobiontics	20	18
Phytohelmints Non-specific pathogenic	20	21
Specific pathogenic	52	41
TOTAL	179	125

ANNEX 1

Class: Nematoda rudolphi, 1808
Subclasses: Adenophorea (v. Linstow, 1905) Chitwood, 1950
Order: Chromadorida Chitwood, 1933
Family: Plectidae Oerley, 1880
Genus: *Plectus* Bastian, 1865
1. P. *granulosus* Bastian, 1865
2. P. *cirratus* Bastian, 1865
3. P. parietinus Bastian, 1865
4. P. *thornei* Ruhm, 1956

Genus: *Proteroplectus* Paramonov, 1964
5. *Proteroplectus parvus* (Bastian, 1865) Pramonov, 1964

Genus: *Wilsonema* Cobb, 1913
6. *Wilsonema auriculatum* (Butschli, 1873), 1913
7. *W. othphorum* (de Man, 1880) Cobb, 1913

Family: Monchysteridae Oerley, 1880
Genus: *Monhystera* Bastian, 1865
8. *M. villosa* Butschli, 1875
9. *M. dolichura* de Man, 1876
10. *M. vulgaris* de Man, 1880

Order: Enoplida Chitwood, 1933
Family: Tripylidae Oerley, 1880
Genus: *Triphyla* Bastian, 1865
11. *T. affinis* de Man, 1880
12. *T. arenicola* de Man, 1880
13. *T. cornuta* Skwarra, 1921

Genus: *Paratripyla* Brzeski, 1964
14. *P. minuta* Brzeski, 1964

Genus: *Prismatolaimus* de Man, 1880
15. *P. dolichurus* de Man, 1880
16. *P. intermedius* (Butschli, 1973) de Man, 1880

Genus: *Tobrilus* Andrassy, 1959
17. *T. leptosoma* (de Man, 1880) Andrassy, 1959

Family: Ironidae de Man, 1876
Genus: *Ironus* Bastian, 1865
18. *I. longicaudatus* de Man, 1884
19. *I. papuanus,* Daday, 1889

Family: Alaimidae Micoletzky, 1922
Genus: *Alaimus* de Man, 1880
20. *A. primitivus* de Man, 1880

Family: Mononchidae Chitwood et Chitwood 1937
Genus: *Mononchus* Bastian, 1865
21. *M. papillatus* Bastian, 1865

Genus: *Myconchulaus* (Cobb, 1916) Andrassy, 1958
22. *M. brachyurus* (Butschili, 1875) Alther, 1954
23. *M. prachyurus* Thorne, 1924
24. *M. solus* Mylvey, 1961

Genus: *Prionchulus* (Cobb, 1971) Andrassy, 1958

87. *L. nirulai* Siddigi, 1965
88. *L. tradicauda* Mersheevskaya, 1951
89. *L. spp.*

Genus: *Xiphinema* Cobb, 1913
90. *X. americanum* Cobb, 1913
91. *X. diversicaudatum* (Micol; 1927) Thorne et Swanger, 1936
92. *X. ensiculiferum* (Cobb, 1893) Thorne, 1937
93. *X. cubensis* Razjivin, 1973
94. *X. spp.*

Family: Diphtheroporidae Thorne, 1935
Genus: *Diphtherophora* de Man, 1880
95. *D. brevicolle* Thorne, 1939

Family: Trichodoridae Thorne, 1935
Genus: *Trichodorus* Cobb, 1931
96. *T. christei* Allen, 1957
97. *T. primitivus* (de Man, 1876) Micoletzky, 1922
98. *T. spp.*

Subclass: Secernentea (Von Linstow, 1905) Dougherty, 1958
Order: Rhabditida Chitwood, 1933
Family: Rhabditidae Oerley, 1880
Genus: *Rhabditis* Dyjardin, 1845
99. *Rh. brevispina* (Claus, 1862) Butschli, 1873
100. *Rh. terricola* Dyjardin, 1845

Genus: *Pelodera* Schneider, 1866
101. *P. Teres* (Schneider, 1866) Dougherty, 1955

Family: Diplogasteridae Steiner, 1929
Genus: *Mesodiplogaster* Goodey, 1963
102. *M. lheritieri* (Mapus, 1919) Goodey, 1963

Family: Cephalobidae Chitwood et Chitwood, 1934
Genus: *Chiloplacus* Thorne, 1937
103. *Ch. demani* (Thorne, 1921) Thorne, 1937
104. *Ch. propinguus* (de Man, 1921) Thorne, 1937
105. *Ch. symmetricus* (Thorne, 1925) Thorne, 1937

Genus: *Cephalobus* Bastian, 1865
106. *C. persegnis* Bastian 1865
107. *C. parvus* Thorne, 1937
108. *C. termophilus* Meyl, 1953

Genus: *Eucephalobus* Steiner, 1936
109. *E. elongatus* (de Man, 1880) Thorne, 1937
110. *E. laevis* Thorne, 1937
111. *E. latus* (Cobb, 1906) Thorne, 1937
112. *E. oxyuroides* (de Man, 1876) Steiner, 1936
113. *E. pulchery* Loof, 1965
114. *E. striatus* (Bastian, 1865) Thorne, 1937
115. *E. teres* Thorne, 1937

Genus: *Acrobeles* von Linstow, 1877
116. *A. cornis* Thorne, 1925
117. *A. otenocephalus* Thorne, 1925

Genus: *Acrobeloides* Cobb, 1924

118. *A. apiculatus* Thorne, 1937
119. *A. buetchli* (de Man, 1880) Steiner et Buhrer, 1937
120. *A. tricornis* (Thorne, 1925) Thorne, 1937

Genus: *Placordira* Thorne, 1937

121. *P. lobata* Thorne, 1937

Family: Panagrolaimidae (Thorne, 1937) Paramonov, 1956

Genus: *Panagrolaimus* Fuchs, 1930

122. *P. rigidus* (Schneider, 1866) Thorne, 1937
123. *P. subelongatus* (Cobb, 1914) Thorne, 1935

Genus: *Panagrodontus* Thorne, 1935

124. *P. armatus* Thorne, 1937

Order: Tylenchida Thorne, 1949

Family: Aphelenchidae Steiner, 1949

Genus: *Aphelenchus* Bastian, 1865

125. *A. avenae* Bastian, 1865
126. *Aph. eremitus* Thorne, 1961

Genus: *Paraphelenchus* Micoletzky, 1925

127. *P. bastili* Das, 1960
128. *P. myceliophtorus* Goodey, 1958
129. *P. pseudoparietinus* Micoletzky, 1925
130. *P. tritici* Baranovskaha

Family: Aphelenchoididae (Fuchs, 1937) Paramonov, 1953

Genus: *Aphelenchoides* Fischer, 1894

131. *A. asterocaudatus* Das, 1960
132. *A. bessey* Christei, 1942
133. *A. bicaidatus* (Immamura, 1931) Filipjew et Sth, 1941
134. *A. helophilus* (de Man, 1880) Goodey, 1963
135. *A. cosposticola* Franklin, 1957
136. *A. limberi* Steiner, 1936
137. *A. saprophilus* Franklin, 1957
138. *A. subparietinus* Sanwal, 1961
139. *A. subtenuis* (Cobb, 1926) Steiner et Buhrer, 1932
140. *A. parietinus* (Bastian, 1865) Franklin, 1955

Family: Tylenchidae Oerly, 1880

Genus: *Tylenchus* Bastian, 1865

141. *T. davainei* Bastian, 1865
142. *T. (Filenchus) filiformis* Butschili, 1873
143. *T. (Filenchus) exiguus* Thorne, 1968

Genus: *Ditylenchus* Filipjev, 1934

144. *D. intermedius* (de Man, 1880) Filipjev
145. *D. myceliogphagus* Goodey, 1958

Genus: *Psilenchus* de Man, 1921

146. *P. hilarulus* de Man, 1921

Genus: *Trophurus* Loof, 1956

147. *T. sculptus* Loof, 1956
148. *T. roigi* Razjivin, 1973

Genus: *Tetylenchus* Filijev, 1936

149. *T. joctus* Thorne, 1949

Family: Hoplolaimidae (Filipjev, 1941)(Weiser, 1953)

Genus: *Hoplolaimus* Daday, 1905
150. *H. indicus* Sher, 1963
Genus: *Rotylenchus* Filipjev, 1934
151. *R. robustus* (de Man, 1876) Filipjev, 1936
Genus: *Helicotylenchus* Steiner, 1945
152. *H. dihystera* (Cobb, 1893) Sher, 1961
153. *H. digonicus* Perry, 1959
154. *H. egytiensis* Tarjan, 1964
155. *H. ethrinae* (Zimmermann, 1904) Goodey, 1950
156. *H. californicus* Sher, 1966
157. *H. canadiensis* Waseen, 1961
158. *H. crenacauda* Sher, 1966
159. *H. lobus* Sher, 1966
160. *H. multicinctus* (Cobb, 1893) Golden, 1956
161. *H. pseudorobustus* (Steiner, 1914) Golden, 1956
162. *H. rotusus* Siddigi et Brown, 1964
163. *H. reynosus* Razjivin, 1973
164. *H. sacchari* Razjivin, 1973
165. *H. varicaudatus* Yuen, 1964
166. *H. vulgaris* Pick et Yuen, 1964
Genus: *Tylenchorhynchus* Cobb, 1913
167. *T. brachycephalobus* Litvinova, 1946
168. *T. capitatus* Allen, 1955
169. *T. cilindricus* Cobb, 1913
170. *T. nudus* Allen, 1955
171. *T. obscurus* Allen, 1955
172. *T. maximus* Allen, 1955
Family: Pratylenchidae Thorne, 1949
Genus: *Pratylenchus* Filipjev, 1934
173. *P. pratensis* (de Man, 1885) Filipjev, 1936
174. *P. brachyurus* (Godfrey, 1929) Goodey, 1951
175. *P. goodeyi* Sher, et Allen, 1953
176. *P. coffeae* (Zimmermann, 1896) Goodey, 1951
177. *P. hexincisus* Tayloer et Lenkins, 1957
178. *P. irregularis* Loof, 1960
179. *P. minyus* Sher et Allen, 1953
180. *P. penetrans* (Cobb, 1917) Filipjev, et Stekhove, 1941
181. *P. scribnueri* Steiner, 1943
182. *P. thornei* Sher et Allen, 1953
183. *P. tumidiceps* Merzheevskaya, 1951
184. *P. vulmus* Allen et Jensen, 1951
185. *P. zeae* Grahm, 1951
186. *P. cubensis* Razjivin et O'Relly, 1976
Genus: *Zygotylenchus* Siddigi, 1963
187. *Z. biterminalis* sp. nov.
Genus: *Radopholus* Thorne, 1949
188. *R. similis* Cobb, 1893) Thorne, 1949
Genus: *Rotylenchulus* Linford et Oliveira, 1940
189. *R. reniformis* Linford et Oliverira, 1940

Family: Heteroderidae Filipijev, 1934
Genus: *Meloidogyne* Goeldi, 1887
190. *M. javanica* (Trueb, 1885) Chitwood, 1949
191. *M. incognita* (Kofoid and White, 1919) Chitwood, 1949
Family: Criconematidae Thorne, 1949
Genus: *Macroposthonia* de Man, 1880
192. *M. rusticum* (Micoletzky, 1915) Taylor, 1936
Genus: *Paratylenchus* Micoletzky, 1922
193. *P. hamatus* Thorne et Allen, 1950
194. *P. elachistus* Steiner, 1949

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ANALISIS ECOTAXONOMICO DE LOS NEMATODOS DE LA CAÑA DE AZUCAR *(SACCHARUM SPP.* HIBRIDO) EN CUBA

Jose P. O'Relly y A. A. Razjivin

RESUMEN

Los resultados del análisis ecotaxonómico de los nemátodos de la caña de azucar en Cuba demuestran el establecimiento de 194 especies que se clasifican dentro de 2 subclases, 5 ordenes, 25 familias y 76 generos.

Con respecto a las especies, se puede observar que el mayor numero de estas pertenece a los ordenes Tylenchida y Dorylaimida (70 a 71 especies); estas son seguidas por las que pertenecen a las ordenes Rhabditida (26), Enoplida (17) y Chromadorida (10).

De acuerdo con la clasificacion ecologica, todas las especies registradas se distribuyen en la siguiente forma: Pararizobióntico con 83 especies (43%); Eusaprobionticos con 4 especies (2%); Devisaprobionticos con 35 especies (18%) y Fitohelmintos con 72 especies (37%).

NEMATODES AND NEMATICIDE TRIALS IN SUGARCANE IN JAVA

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ABSTRACT

A survey carried out during 1976-1978 on 8 sugar factories showed that the nematode genera *Helicotylenchis, Pratlenchus, Meloidogyne* and *Criconemoides* were most common in the soil samples of all the 8 sugar factories examined and *Pratylenchus* and *Meloidogyne* in the root samples. Other nematode genera found were *Hirschmaniella, Aphelenchus, Rotylenchus, Tylenchus, Hemicriconemoides, Trichodorus, Paratylenchus, Xiphinema, Dorylaimus, Ditylenchus, Hoplolaimus, Longidorus, Aphelenchoides, Hemicycliophora* and *Tylenchorynchus.* On the light sandy soils (heaviness 4-5) the nematode population was higher than on the heavier soils (heaviness 6-9).

Nematicide trials carried out on soils with different levels of soil heaviness during 1973-1977 showed that yield responses were obtained on light sandy soils with a soil heaviness of 4-5. Soil fumigation with D-D 180 1/ha (overall application), D-D 280 1/ha (overall application) and Nemagon 30 1/ha (furrow application) increased cane yield with 21.20-23.75%, 16.51-32.89% and 16.49-29.59%, respectively and sugar yield with 18.90-26.57%, 21.07-32.21% and 19.74-30.42% respectively, furrow application with D-D 100 1/ha was promising. Soil fumigation with D-D and Nemagon on the Sf. Ngadirejo sufficiently suppressed the population of plant parasitic nematodes. There was no difference between plant parasitic nematode population in the treated and untreated control plots four (4) months after fumigation.

Soil fumigation with a climax 250 Subsoil Sprayer on a semi practice scale on the Sf. Mgadirejo in 1976/1977 showed that D-D 100 1/ha (furrow application), D-D 180 1/ha and D-D 280 1/ha (overall application) increased cane yield with 15.38%, 23.78% and 26.96%, respectively and sugar yield with 18.90%, 23.79% and 36.92%, respectively.

INTRODUCTION

Since root infestation of sugarcane by *Meloidogyne* was reported for

the first time by Trueb in 1885, the occurrence of nematodes and the effect of nematicides on nematodes and yield in sugarcane were investigated in different countries (Birchfield[1], Carbonell[2], Dick[3], Hu et al[7], O'Relly and Perez Milian[8], Sandhu and Behar[9], Sing and Misa[10]).

Almost a century passed before Handojo, Siswojo and Legowo[4,5,6,11,12] investigated the occurrence of nematodes in Javan sugarcane fields and carried out nematicide trials.

MATERIALS AND METHODS

Survey

Of the 59 sugar factories in Indonesia, 56 are located in Java viz. 33 in East Java, 16 in Centre Java, 7 in West Java, and 3 on the outer islands.

Sugarcane in Java is mostly grown on heavy clay soils. Light sandy soils are mostly found in some parts of East Java.

The first phase of surveys on nematodes (1976-1978) was conducted in 7 sugar factories in East Java and 1 sugar Factory in Yogyakarta region following the stratified sampling techniques.

Each sugar factory comprises 10 cane fields @ 1 ha, except for one factory with 4 fields.

Soil sampling was done with a 2.5 cm diameter soil sampling tube inserted 25 cm deep in the soil. From each field 100 soil samples and 100 root samples were collected. For the year 1976-1977 soil samples were taken at 4 intervals, namely; before planting, canes were 2-3 months, 6-7 months and 10-11 months old. Root samples were taken during the last two intervals. For the year 1977-1978 the samples were collected at 3 periods.

A total of 880 soil and 660 root samples were processed. Nematodes were isolated by Oostenbrink elutriator method using 100 ml soil samples and 5 g root samples. Root samples were processed using a blender for 20 seconds at 23,000 rpm. Observations were done twice with 10 ml suspension.

Nematicide Trials

To investigate the effectiveness of soil fumigation and nematicides on cane soils with different levels of soil heaviness, trials were carried out at the Sf. Ngadirejo, Sf. Cukir, Sf. Asembagus, S. Ceper Baru, BP3G substation at Sf. Jatiroto, Sf. Cepiring and the Indonesian Sugar Experiment Station (BP3G) with a soil heaviness of 4, 4.5-5, 6.5, 6-6.5, 6.5,7-9 and 8-9, respectively.

Nematicides tested during 1973-1977 were soil fumigants D-D at the following rate of applications (100 1/ha furrow application; 180 1/ha and 280 1/ha overall application; Nemagon at 20 1/ha and 30 1/ha furrow application),

Vapam at 400 1/ha overall application. The granular nematicides tested were Temik at the following rate 22 kg, 55 kg and 75 kg/ha; Nemacur at 50 kg, 75 kg and 100 kg/ha; Terracur at 45 kg/ha; Mocap at 22.5 kg and 45 kg/ha; Furadan at 2 kg and 4 kg a.i./ha and CGA at 29 kg and 40 kg/ha.

Fumigants and Nematicide Application

Soil fumigation was done after the soil was ploughed and levelled. These operations were done with a tractor at the sugar factories; Ngadirejo, Jatiroto, Assembagus and Cepiring. Land preparation at other sugar factories were made using animal drawn implements. The soil was fumigated on an overall bases 4-5 weeks before planting with the aid of the Shell Soil Fumigant Injector, at a depth of 20 cm and in 30 cm squares by staggering the position in adjacent rows. Furrow applications were carried out along the furrow (30 x 100 cm).

The granular nematicides were applied at planting time or one day after planting, except Temik which was applied 6 weeks after planting. The nematicides were evenly distributed in a small ditch made along the furrow: the ditches at both sides of the cuttings when the cuttings were planted in the middle of the furrow or one ditch when the cuttings were planted at the border of the furrow.

Determination of Nematode Population

Soil sampling was done with 2.5 cm diameter soil sampling tube inserted 25 cm deep in the soil. Ten borings were taken from each plot and these were standardized to 400 g samples. One hundred gram soil was processed using Oostenbrinks elutriator method. The nematode suspension was standardized to 100 ml for each sample and the nematodes were counted in 10 ml. suspension. The total number of nematodes per 400 g soil was then computed. The design of the trials was a randomized block with 5.6 replications. Plot size was used as usual at the respective sugar factories (20-25 furrows @ 200 cm), at the Sugar Experiment Station one plot consisted of 5 furrows at 6 m. The varieties used were Ps 41, POJ 3016 and POJ 2878.

D-D Soil Fumigation with Climax 250

The trial was conducted in light sandy soils of the Sf. Ngadirejo, where crop rotation with leguminous plants *(Crotollaria anagyroides, Calopogonium mucunoides* and *Centrosema pubescens)* was practiced.

Land preparation was done as follows: the green manure plants (14-16 months old) were pulled down and buried by a Caterpillar D4D in March 1976; about 2 months later the soil was ploughed and rotavated with a MU 60 rotavator drawn by IH 624 tractor; afterwards the land was cleaned by raking the remnants of green manure plants which were not yet rotten; and 1-2 weeks later rotavated for the second time.

Soil fumigation with the Climax 250 Subsoil Sprayer was done one

TABLE 1. Average number of nematodes in 100 ml soil/5 g Root samples

Sugar factory	Ngadi-rejo	Asem-bagus	Madu-kismo	Pesan-tren	Lestari	Krebet Baru	Kebon-agung	Candi
Soil heaviness	4	4-5-6	4-5-6	4-5-6	5-6-7	6-7	6-7-5	7-8-9
GENERA Nematode in soil samples								
Helicotylenchus	1	138	67	46	58	56	18	20
Pratylenchus	9	12	23	16	16	12	6	1
Meloidogyne	12	14	17	7	4	5	3	3
Criconemoides	37	65	16	16	11	10	5	2
Hirchmaniella	–	–	2	1	–	–	–	–
Aphelenchus	12	7	12	5	4	–	–	2
Rotylenchus	5	2	6	2	3	–	2	2
Tylenchus	5	9	28	15	15	1	4	9
Hemicriconemoides	–	–	14	1	3	–	–	–
Trichodorus	–	–	1	1	–	–	–	–
Paratylenchus	–	–	1	–	–	–	–	–
Xiphinema	–	2	2	–	1	2	–	–
Dorylaimus	–	–	3	–	–	–	–	–
Ditylenchus	–	–	1	1	–	–	–	–
Hoplolaimus	1	–	3	–	5	1	–	–
Longidorus	–	1	–	–	–	1	–	–
Aphelenchoides	–	–	–	1	1	–	–	–
Hemicycliophora	–	–	1	2	1	–	6	–
Tylenchorynchus	–	–	–	–	–	10	1	1
Others	2	–	1	–	–	–	–	–
Total	84	250	198	102	122	98	45	40
Root samples								
Pratylenchus	65	28	75	112	41	43	81	11
Meloidogyne	77	639	38	12	4	4	2	2
Hoplolaimus	–	2	1	–	1	–	–	–
Total	142	669	114	124	46	47	83	13
Total number in soil and root samples	226	919	312	226	168	145	128	53

month later. The Climax Subsoil Sprayer 250 has an hourly capacity of up to 1.25 ha, a working width of 2.5 meters; contents liquid tank of 250 1, and the number of shears is 5; power of 60 HP and up is required; pressure is 5-6 atu and injection is done at a depth of 15 to 20 cm. The canes were planted in July 1976, about one month after the fumigation. The canes were harvested 12.5-15 months.

The trials compared are the treated Nemagon 30 1/ha and D-D 100 1/ha furrow application; D-D 180 1/ha and D-D 280 1/ha overall application and non-treated controls. Plot size was 2 ha; number of replications 3. Variety used was POJ 3016.

RESULTS

Survey

The plant parasitic nematodes predominantly observed as can be seen in Fig. 1 and 2 and Table 1 were *Helicotylenchus sp., Pratylenchus sp., Meloidogyne sp., Criconemoides sp.,* from samples in 8 sugar factories examined. *Pratylenchus* and *Meloidogyne sp.* were common in root samples.

Other nematodes observed in lesser population were *Hirchamniella* sp., *Aphelenchus* sp., *Rotylenchus* sp., *Tylenchus* sp., *Hemicriconemoides* sp., *Trichodorus* sp., *Partylenchus* sp., *Xiphinema* sp., *Dorylaimus* sp., *Ditylenchus* sp., *Hoplolaimus* sp., *Longidorus* sp., *Aphelenchoides* sp., *Hemicycliophora* sp., and *Tylenchorynchus* sp.

The highest populations of nematodes were found on the light soils of the Sf. Asembagus (Besuki region) in the most eastern part of East Java and the Sf. Madukismo in Yogyakarta region (250 and 198 per 100 ml soil and 669 and 114 per 5 g roots resp.); moderate populations at the Sf. Lestari, Sf. Ngadirejo. Sf. Pesantren (Kediri region) and Sf. Krebet Baru (Malang region); and the lowest nematode populations were found at the Sf. Kebonagung (Malang region) and Sf. Candi (Surabaya region).

Nematode population on the light sandy soils (heaviness 4-5) was higher than on the heavier soils.

It seems that there is a correlation between the number of nematodes with rainfall in the wet season: in soil samples the number of nematodes increased from the beginning to the middle of the wet season, while in root samples the nematode population increased rapidly from the middle to the end of the wet season; then both decreased in the dry season.

Nematicide trials

The treatments which gave significant increases in yield in the nematicide trials 1973-1977 are given in Table 2.

FIGURE 1. Distribution of nematode populations from soil samples

FIGURE 2. Distribution of nematode populations from root samples

TABLE 2. Treatments with significant yield increases in nematicide trials 1973-1977

Location	Soil heaviness	Nematicide Rate per ha	% Difference with control: Cane Yield 1973/1974	Cane Yield 1974/1975	Cane Yield 1975/1976	Cane Yield 1976/1977	Sugar Yield 1973/1974	Sugar Yield 1974/1975	Sugar Yield 1975/1976	Sugar Yield 1976/1977
Sf. Ngadirejo	4	D-D 180 l	21.20[2]	23.75[1]	0.91	–	18.90[1]	26.57[1]	−0.09	–
		D-D 280 l	32.89[2]	18.34	16.51[1]	–	33.21[2]	16.78	21.07[2]	–
		D-D 100 l	1.10	24.59[1]	–	–	−1.22	26.22[1]	–	–
		Nemagon 30 l	16.49[1]	29.59[2]	8.94	–	19.74[1]	30.42[2]	9.48	–
		Nemacur 75 kg	–	–	6.42	–	–	–	13.31[1]	–
		Furadan 4 kg a.i.	–	–	13.76[1]	–	–	–	12.93[1]	–
Sf. Cukir	4.5 - 5	D-D 280 l	8.84	–	–	–	10.99[1]	–	–	–
		Temik 55 kg	8.57	2.07	−7.18	–	10.90[1]	−0.04	−6.64	–
BP 3G	8 - 9	Furadan 2 kg a.i.	–	–	12.29[1]	−1.73	–	–	11.88[1]	1.33

1) t 0.05 *2) t 0.01*

Table 2 shows that the nematicide especially D-D and Nemagon increased both cane and sugar yield significantly on light sandy soils with a soil heaviness of 4-5 (Sf. Ngadirejo, Sf. Cukir).

Soil fumigation with D-D 180 1/ha (overall application) and Nemagon 30 1/ha (furrow application) increased cane yield with 21.20-23.75%, 16.51-32.89% and 16.49-29.59%, respectively, and sugar yield with 18.90-26.57%, 21.07-

TABLE 3. Number of nematodes/400 g soil in trial 1974/1975 at Sf. Ngadirejo

Soil[1] sample No.	Nematicide kg/1 per ha									
	Control	D-D 180 1	D-D 280 1	D-D 100 1	Nemagon 30 1	Temik 55 kg	Temik 75 kg	Nemacur 50 kg	Mocap 22.5 kg	Mocap 45 kg
1	1700	2436	2324	1372	1992	2172	1444	1488	2004	1676
2	1544	352	168	720	1084	1580	1692	1608	1784	1516
3	416	68	48	92	108	184	232	148	148	168
4	476	540	436	504	180	492	324	356	336	460
5	424	592	596	488	428	400	416	440	560	408
6	1252	780	648	692	1016	944	1068	796	916	872
7	968	872	964	1508	1588	1076	1336	652	888	1008

1) Soil samples taken one day before fumigation (No. 1), 3 weeks, after fumigation (No. 2) and when the cane was ±6 weeks (No. 3), ±3 months (No. 4), ±6 months (No. 5), ±9 months (No. 6) and ±12 months (No. 7) old.

TABLE 4. Results of demoplot soil fumigation with climax 250 subsoil sprayer at Sf. Ngadirejo 1976/1977

Treatment	Cane Yield		Sugar yield	
	q/ha	% Difference	q/ha	% Difference
Nemagon 30 1/ha (furrow)	1032.3	−3.25	117.90	−3.13
Control	1067.0	–	121.71	–
D-D 100 1/ha (furrow)	952.7	+15.38	107.06	+18.90
Control	825.7	–	90.03	–
D-D 180 1/ha (overall)	1053.7	+23.78	116.87	+23.79
Control	851.3	–	94.41	–
D-D 280 1/ha (overall)	956.0	+26.96	106.62	+36.92
Control	753.0	–	77.87	–

33.21% and 19.74-30.42% respectively.

Furrow application with D-D 100 1/ha was promising.

Research on the influence of the granular nematicides Nemacur, Furadan and Temik is still needed.

Nematode observations of the nematicide trial 1974/1975 (Table 3) showed that soil fumigation D-D and Nemagon at the Sf. Ngadirejo sufficiently suppressed the population of plant parasitic nematodes, but when the cane was 3 months old, i.e. $\pm$4 months after the fumigation was done, the nematode population in the fumigated plots increased greatly. There was no difference in the population of amount of nematodes in both treated and control plots.

The plant parasitic nematodes observed in the nematicide trials 1974/1975 were *Criconemoides* sp., *Helicotylenchus* sp., *Meloidogyne* sp., *Pratylenchus* sp., *Aphelenchus* sp., *Hoplolaimus* sp., *Dorylaimus* sp., *Tylenchus* sp., *Trichodorus* sp., *Xiphinema* sp., *Tylenchorhynchus* sp., *Rotylenchus* sp., *Paratylenchus* sp., and *Hirchmaniela* sp.

D-D soil fumigation with the Climax 250 subsoil sprayer

The result of the nematicide trials 1973-1977 and on preliminary testing, in 1976/1977 soil fumigation trials with Nemagon and D-D on a semi-practice scale that were carried out in Sf. Ngadirejo using the Climax 250 subsoil sprayer are given in Table 4.

The results are in agreement with the previous nematicide screening trials: D-D 1/ha (furrow application), D-D 180 1/ha and D-D 280 280 1/ha (overall application) increased cane yield with 15.38%, 23.78% and −26.96%, respectively and sugar yield with 18.90%, 23.79% and 36.92%, respectively. Only furrow application with Nemagon 30 1/ha did not give positive results.

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ESTUDIOS DE NEMATODOS Y NEMATICIDAS EN CAÑA DE AZUCAR EN JAVA

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RESUMEN

Un catastro llevado a cabo durante el periodo de 1976-1978 en 8 factorias azucareras mostró que los generos de nematodos *Helicotylenchus, Pratylenchus, Meloidogyne* y *Criconemoides* eran los mas comunes en las muestras de suelo y *Pratylenchus* y *Meloidogyne* en las muestras de raices de las ocho factorias. Otros generos de nematodos encontrados fueron: *Hirchmaniella, Aphelenchus, Rotylenchus, Tylenchus, Hemicriconemoides, Trichodorus, Paratylenchus, Xiphinema, Dorylaimus, Ditylenchus, Hoplolaimus, Longidorus, Aphelenchoides, Hemicycliophora* y *Tylenchorynchus.* En los suelos livianos arenosos (peso de 4-5) los poblaciones de nematodos fueron mas altas que en los suelos mas pesados (peso de 6-9).

Pruebas con nematicida llevados a cabo en suelo con diferentes

niveles en peso durante el periodo de 1973-1977, demostraron que las respuestas en produccion en los suelos livianos arenosos con un peso de 4-5. La fumigacion de suelo con D-D 180 1/ha (aplicacion total), D-D 280 1/ha (aplicacion total) y Nemagon 30 1/ha (aplicacion en el zurco) aumentaron la produccion de caña a un 21.20 - 23.75%, 16.51 - 32.89% y 16.49 - 29.59% respectivamente y aumentaron la produccion de azucar 18.90 - 26.57%, 21.07 - 33.21% y 19.74 - 30.43% respectivamente, la aplicacion en el zurco de D-D 100 1/ha fue prometedora. La fumigacion de suelo con D-D y Nemagon en Sf. Ngadirejo redujo considerablemente las poblaciones de los nematodos parasiticos, pero cuatro meses despues de la fumigacion haberse efectuado no existia diferencia entre las parcelas tratadas y las no tratadas.

La fumigacion del suelo con una asperjadora de subsuelo Climax 250 en una escala semi-comercial en Sf. Ngadirejo en el periodo de 1976/1977 mostró que el D-D 100 1/ha (aplicacion en el zurco), D-D 180 1/ha y D-D 1/ha (aplicacion total) aumentaron la produccion de caña en un 15.38%, 23.78% y 26.96% y la produccion de azucar en un 18.90%, 23.79% y 36.92%, respectivamente.

SUGARCANE VARIETIES RESISTANT TO *MELOIDOGYNE INCOGNITA* CHITWOOD, 1949

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ABSTRACT

Resistance of ten sugarcane varieties to the root knot nematode, *M. incognita* was tested.

The sugarcane variety, My 6422 proved to be resistant with only ten galls.

The seven varieties, My 5715, C 334-64, My 54129, B 60267, Ja 64-16, C 79-77 and Ja 64-20, were tolerant with only 11 to 20 galls. The variety Ja 60-19 was susceptible, exhibiting 21 galls. My 5753 was the only highly susceptible variety exhibiting 31 galls per sample.

In all 10 varieties root galls or knots were produced and the nematode completed its life cycle. None of the varieties is immune to *M. incognita.*

No significant differences were found between the control and the affected varieties either in root weight or stalk length.

INTRODUCTION

Nematodes are important in tropical countries and cause heavy economic losses in nearly all cultivated plants. Sugarcane, our main economic crop is not an exception.

The sugarcane varieties show a specific susceptibility to specific nematodes. This could be one of the reasons for the low yield of sugarcane varieties. Its specific effects were not studied until recently. However, Good[1] in 1949 and Holtzman[2] in 1964 stated that nematodes, as a whole, may reduce cane yield by over 4%.

O'Relly[3] in 1972 tested the resistance of the main varieties cultivated in Cuba. He concluded that varieties POJ 2878, B 4372, C 236-51, C 87-51, CG 127-45, PR 980, My 5464, My 5514 and Ja 60-5 were susceptible to nematodes.

Agrotechnical methods including the use of resistant varieties are highly effective under our environment. Selected varieties were tested to evaluate their resistance to the most devasting parasitic species *M. incognita* in the Cuban sugarcane crop.

MATERIALS AND METHODS

In this trial, 10 sugarcane varieties in the enlargement stage were used: My 5715, C 334-64, My 54129, My 5723, B 60267, Ja 64-11, My 6422, Ja 64-19, C 79-67 and Ja 64-20.

Potted soil, autoclaved at 50°C for 2 hr, was infested with cut galled roots of *Cucurbita pepo* infected with *Meloidogyne incognita.* Twenty grams of galled roots was used per pot. One bud cutting of each cane variety was planted separately to each plot. To each pot *C. pepo* was also planted to check the effectiveness of the nematode inoculum as determined by microscopic observation of females, oathecae and larvae of the nematode and root gall formation.

The degree of infestation and varietal resistance were determined by taking 1 g root sample and counting the knots. When the root system was about 1 g, three samples were taken at random and the average was taken from the last two samplihgs.

TABLE 1. Evaluation of the degree of infestation and resistance to *M. incognita* was based on the following scheme

No. of knots per g root sample	Degree of infestation	Degree of resistance
0	0	Immune
1 – 10	1	Resistant
11 – 20	2	Tolerant
21 – 30	3	Susceptible
30 or more	4	Highly susceptible

Sampling was done every 15 days until 45 days. For each sampling the knots, females oothecae and adults per variety were recorded. Germination was counted until the 30th day while stalk measurement was done until the end of the trial.

RESULTS AND DISCUSSION

There were no significant differences in germination among the infested

plants. Germination was over 80% for all varieties in the three trials.

The first trial showed that varieties C 334-64, My 54129, My 5724, Ja 64-11, My 6422, Ja 64-19, and C 79-77 were being attacked by young females with knot formation. Although oothecae were found, the life cycle was probably not completed due to the short time (15 days). However, in My 5715, B 60267 and Ja 64-20 varieties, not even larva was found.

Root systems of variety Ja 64-20 showed no difference between the infested plant root system and that of the control (Fig. 1). During the first trial, this variety was not attacked by *M. incognita*. C 334-64 showed differences between the root system and the length of the stalk of the inoculated and control plants. After 30 days, the second trial, all varieties have been infested by adults and young females, also larvae and oothecae found as well as greater knots. Thus, we think *M. incognita* is capable of completing its whole life-cycle in each of the varieties tested, laying aside the possibility of having immune varieties.

FIGURE 1. Root systems of infested and central plants, Ja 64-20

FIGURE 2. Variety My 5723 had 35 nodes, 50 females and 23 oothecae.

FIGURE 3. Variety Ja 64-22 had 6 nodes, 10 females and 5 oothecae.

Data taken from the last sampling are shown in Table 2.

TABLE 2. Evaluation of varietal resistance to *M. incognita*

Variety	No. of nodes in sampling			Average	Degree of Infestation	Varietal resistance
	First Trial	Second Trial	Third Trial			
My 5715	–	6	20	13	2	Tolerant
C 334-64	*	16	20	18	2	Tolerant
My 54129	*	13	17	15	2	Tolerant
My 5723	*	35	27	31	4	Highly susceptible
B 60267	–	14	19	16	2	Tolerant
Ja 64-11	*	17	23	20	2	Tolerant
My 6422	*	6	14	10	2	Tolerant
Ja 64-19	*	21	24	22	3	Susceptible
C 79-67	*	12	27	19	2	Tolerant
Ja 64-20	–	13	9	11	2	Tolerant

* *Knots formation*
– *Knots do not exist*

My 5723 was highly susceptible with an average of 31 knots, 66 adult females and 13 oothecae. In contrast, My 6422 was found to be highly resistant variety having only 10 knots, 15 females and 5 oothecae.

Variety Ja 64-19 was susceptible to *M. incognita* having 21 knots, 51 females and 10 oothecae. The other varieties, My 5715, C 334-64, My 54129, B 60267, Ja 64-11, C 79-77 and Ja 64-20, showed diseases rating of 2, and showing resistance with 11 to 20 galls.

Seven out of 10 varieties tested may be used for commercial planting in the future with an acceptable resistance to *M. incognita.*

So far, there was no immune variety. The results showed that 70% of the varieties tested were found to be tolerant and 20% resistant, My 5723 was highly susceptible and Ja 64-19, susceptible.

There were no significant differences between the control and the affected plant either in the root weight or in the stalk length (Table 2).

TABLE 3. Analysis of covariance

	Stalk length average (cm)	Root weight average (g)
Affected varieties	20.2	5.4
Sampling	21.2	6.2
Difference	1.0	0.8
T Calculated	0.713	0.785
T Theoretical	2.105	2.105
Significant at 5% level	0	0

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VARIEDADES DE CAÑA DE AZUCAR RESISTENTES A *MELOIDOGYNE INCOGNITA* CHITWOOD, 1949

J.P. O'Relly Legón y A. A. Razjivin

RESUMEN

La resistencia en diez variedades de caña de azúcar al nematodo nodular *M. incognita* fue estudiada.

La variedad de caña de azucar My 6422 probo ser resistente, solo diez nodulos fueron encontrados en la muestra estudiada.

Las siete variedades, My 5715, C 334-64, My 54129, B 60267, Ja 64-16, C 79-77, Ja 64-20, demonstraron ser tolerantes encontrandose

exhibiendo 31 nodulos por muestra.

En todas las variedades el nematodo indujo la formacion de nodulos y completo su ciclo de vida. Ninguna de las variedades es inmune a *M. incognita*.

No se encontraron diferencias significativas entre las muestras de las variedades afectadas tanto en el peso de la raiz como en el largo del tallo.

INFLUENCE OF DISEASES AND HERBICIDES ON SUGARCANE GROWTH IN THE GREENHOUSE

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ABSTRACT

Infection with strain H of the sugarcane mosaic virus (SCHMV H) or with ratoon stunting disease (RSD) reduced sugarcane growth significantly, as did exposure to the herbicides fenac [(2,3,6-trichlorophenyl) acetic acid] and terbacil (3-*tert*-butyl-5-chloro-6-methyluracil). However, the combined effects of SCMV-H and herbicides or RSD and herbicides did not cause greater yield losses than the individual effects of either disease or herbicides. The possibility that herbicides were "curing" the disease did not seem likely because mosaic and RSD symptoms did not diminish in herbicide-treated plants.

INTRODUCTION

Sugarcane mosaic and ratoon stunting disease (RSD) are two major sugarcane diseases in Louisiana, as well as in many other areas of the world (Abbott[1] Koike[5], Steindl[7]).

Herbicides have been reported to either increase or decrease root disease severity in some plants (Grau and Reiling[4]). Little is known however of the effects of herbicides on the severity of sugarcane diseases.

The herbicides fenace [(2.3.6-trichlopophenyl) acetic acid] and terbecil (3-*tert*-butyl-5-chloro-6-methyluracil) are used widely in sugarcane in Louisiana for preemergence control of johnsongrass from seed and of many other annual weeds. Under field conditions, sugarcane tolerates herbicide dosages commonly used for weed control. Fenac at 4 kg/ha and terbacil at 2 to 3 kg/ha (Milhollon[6]). Fenac has growth-regulator properties and is toxic to roots at high concentrations. The primary mode of action of terbacil probably is the inhibition of photosynthesis.

The purpose of this study was to determine the effect on sugarcane growth of infection with mosaic or RSD and concomitant treatment with fenac or terbacil.

MATERIALS AND METHODS

Two RSD-herbicide and two mosaic-herbicide experiments were conducted

in the greenhouse during 1975 and 1976. Sugarcane (interspecific hybrids of *Saccharum*) cultivars L 62-96, highly susceptible to injury by RSD, and CP 52-68, highly susceptible to injury by mosaic, were used in the experiments.

In the first RSD-herbicide experiment, cuttings were selected from the first progeny of greenhouse-grown L 62-96 plants that had either been inoculated with RSD by dipping single-bud cuttings in infective cane juice or freed of the disease by hot-water treatment (HHWT, 50°C, 2 hrs). In the second experiment, cuttings were obtained from HWT and RSD-infected field-nursery plants of L 62-96.

For the mosaic-herbicide experiments, cuttings were obtained from greenhouse-grown CP 62-68 plant cane given HWT and free of both mosaic and RSD. Three weeks after planting, shoots in randomly selected pots were inoculated with sugarcane mosaic virus strain H (SCMV-H) by a previously described method (Dean[2], Gillaspie[2]).

In all experiments, cuttings were prepared for planting by cutting stalks into single-bud cuttings and by pregerminating them in an incubator at 35°C for 72 hr. Cuttings were then planted about 2.5 cm deep in 23-cm clay pots filled with 4000 cm^3 of steam-sterilized, Commerce silt loam soil, pH 6.5.

Soon after planting, the pots were sub-irrigated with water until the soil surface was moist. Commercial formulations of fenac (3.4 kg/ha) or terbacil (1.3 kg/ha) were mixed with water and sprayed over the soil surface with an endless-belt spray table calibrated to spray 375 liters/ha. For each experiment, four pots were not sprayed with herbicide to serve as controls.

The pots were placed on greenhouse benches for growth, and fertilized at intervals with a dilute liquid fertilizer containing N, P, K, and trace amounts of Fe and Zn. Pots were watered as needed and as uniformly as possible with a hand-held sprinkler. Treatments that caused stunting of plants were selectively watered to avoid excesses.

The experiments were harvested for dry weight 16-23 weeks after planting and harvested again 16-27 weeks later. At each harvest, shoots were removed at soil level, oil into 15-cm long pieces, and oven-dried at 85°C. The dry weights for both harvests were combined to give one dry weight yield per pot.

The experiments were 2 x 3 complete factorials arranged in a randomized complete block design with four replicates.

RESULTS AND DISCUSSION

Combined data from two experiments with SCMV-H and the herbicides fenac and terbacil are presented in Table 1. The statistical analysis showed that yields were affected by an interaction between SCMV-H and herbicides. Infection with SCMV-H alone reduced yield (dry weight of top growth) significantly below

that of the nontreated, health control, as did treatment with fenac or terbacil. The combination of either herbicide and SCMV-11 did not reduce yields significantly more than did either alone. This lack of an additive effect when plants were both diseased and treated with herbicides suggests that one of the treatments causing injury prevented further injury by the other treatment or that the injury caused by each treatment was reduced proportionately. Apparently the herbicides did not cure the mosaic disease in plants because the typical leaf mottling of mosaic did not diminish in these treatments.

TABLE 1. Individual and combined effects of herbicides and sugarcane mosaic virus strain H (SCMV-H) on growth of sugarcane cultivar CF 52-68

Herbicides and rate (kg/ha)	Dry wt of top growth[1]	
	Healthy	SCMV-H
	g	g
None	75.1	61.0
Fenac – 3.4	64.5	61.2
Terbacil – 1.3	61.2	60.5
LSD 0.05 = 7.4		

[1]*Mean dry weight for two harvests.*

Data from one experiment with ratoon stunting disease (RSD) and fenac and terbacil are presented in Table 2. Although two experiments were conducted with the RSD-herbicide combination, data from the experiment with field-nursery plants are not included because for some unknown reasons, growth in that experiment was much retarded even in the nontreated controls. In this experiment the statistical analysis showed that yields were affected by an interaction between RSD and herbicides. Infection with RSD alone and treatment with fenac or terbacil alone reduced yield significantly below that of the nontreated healthy control.

TABLE 2. Individual and combined effects of herbicides and ratoon stunting disease (RSD) on growth of sugarcane cultivar L 62-96

Herbicides and rate (kg/ha)	Dry wt of top growth[1]	
	Healthy	SCMV-H
	g	g
None	132.9	106.4
Fenac – 3.4	98.8	90.9
Terbacil – 1.3	85.3	98.1
LSD 0.05 = 7.4		

[1]*Mean dry weight for one harvest.*

The combination of either herbicides and RSD did not reduce yields significantly more than did either alone. This interaction is similar to that found for mosaic and herbicides. Here again herbicides apparently did not cure RSD in plants, because the typical nodal symptoms that characterize the presence of RSD were observed at the conclusion of the experiments.

Fenac and terbacil, when applied as recommended, generally do not reduce sugarcane yields under field conditions. The yield reductions observed in these studies probably were caused by the frequent watering that was necessary to sustain plants under greenhouse conditions. Such frequent watering probably caused excessive leaching of the herbicides into the sugarcane root zone.

Although fenac and terbacil are relatively safe herbicides for use in sugarcane fields, injury to sugarcane can occur, particularly on soil of certain textures (Millhollon[6]). This study shows that this injury would probably not be compounded if the sugarcane were also infected with mosaic or RSD.

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LA INFLUENCIA DE LAS ENFERMEDADES Y YERBICIDAS EN EL CRECIMIENTO DE LA CAÑA DE AZUCAR EN EL INVERNACULO

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RESUMEN

La infección por la cepa H del virus mosaico de la caña de azúcar (SCMV-H) o por la enfermedad emanismo del retoño (RSD) redujeron significativamente el crecimiento de la caña de azúcar, al igual que la exposicion a los yerbicidas fenac (2.3.6-trichlorophenyl) acetic acid y ferbacil (3-tert-butyl-5-chloro-6-methyluracil). Sin embargo la combinacion de SCMV-A mas yerbicida o RSD y yerbicida no causa mayor reducción en rendimento que cada una por separado (el efecto de las enfermedades o de los yerbicidas). La posibilidad de que los yerbicidas se puedan usar para curar las enfermedades no parace ser el caso y que los sintomas del mosaico y del RSD no se disminuyeron en las plantas tratadas con yerbicidas.

A METHOD FOR BULK HARVESTING *USTILAGO SCITAMINEA* SYD, SPORES

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ABSTRACT

A method of sugarcane smut spore harvesting has been proposed to allow handling of large quantities of spores in a short period of time.

The method has the following sequence of operations:

a) New smut whips are harvested in the field and taken to a processing room;
b) The leaves and leaf sheaths are taken out exposing the whips for drying. The basal part of the whips, where the spores are not mature, is also eliminated.
c) The whips are spread on shelves of wire screen inside a small drying chamber equipped with an electric air dehumidifier.
d) After 12-16 hours, the spores are dried enough to be collected by a vacuum cleaner (1 Hp motor), provided with a disposable paper bag collector.
e) The collected spores are sifted in a sieve of 100 mesh. The bottom of the sieve is adjusted to a funnel which is connected to the vacuum cleaner tube which greatly facilitates sieving.
f) After a final drying in the laboratory, the spores are stored under 5° C in a desiccator containing silica-gel.

This method greatly reduces the time required for processing the spores as compared to the conventional method that has been used.

Germination tests showed that the viability of the spores collected by this method ranges from 86% to 93 %.

INTRODUCTION

In the State of Sao Paulo, Brazil, every variety to be commercially grown must be approved for smut resistance by the State Commission for the Control of Sugar Cane Smut. This approval is obtained only after four simultaneous field trials each carried out in different States. The cuttings are artificially inoculated by immersion in a standard spore suspension of *ca* 5 x 10^6 spores/ml, with at least 80% germination, for 15 minutes (Figueiredo *et al*[1]). Each institution involved in sugarcane breeding is also engaged in this official testing program, providing land, technical assistance and any other physical and personnel facilities. One of these facilities refers to the provision of spores for these tests. It is estimated that about 3.5 to 4.0 kg of spores/year is necessary to carry out each trial. This estimates does not include the institutions need for their own screening tests.

When the need for large quantities of spores arises, the only method available is the conventional one. This consists of drying the whips on a table under normal room conditions, and after two to three days the whips are scrubbed with a small hard brush to release the spores still attached to them.

Mata[3] and Mata and Tokeshi[4] adopted this method and eventually utilized an air dehumidifier in the room, but they did not mention if the drying efficiency had been increased. Nevertheless, due to the unsatisfactory drying, this method always requires whip scrubbing, resulting in high spore dispersion into the air. Consequently, this method is very inconvenient for the operators, as the dispersed spores causes deep impregnation on the clothes and body, and also allergic reactions to some persons.

In said method, the collected spore mass is processed by a stepwise sieving for gradual elimination of impurities. A 100 or 200 mesh is used for the final sieving. These sieves are easily clogged, requiring the aid of a soft brush to enhance sieving. Even so, this process is very time-consuming and difficult.

Taking into account these difficulties, a method was developed that utilizes a small drying chamber with an air dehumidifier inside, a vacuum cleaner to collect spores and a sieve-funnel set adapted to the vacuum cleaner for final processing.

The method for bulk harvesting of smut spores is described here, accompanied by some measurements that indicate the efficiency of the method.

MATERIALS AND METHODS

Spore collection method

During one spore harvesting process, weights were recorded at different

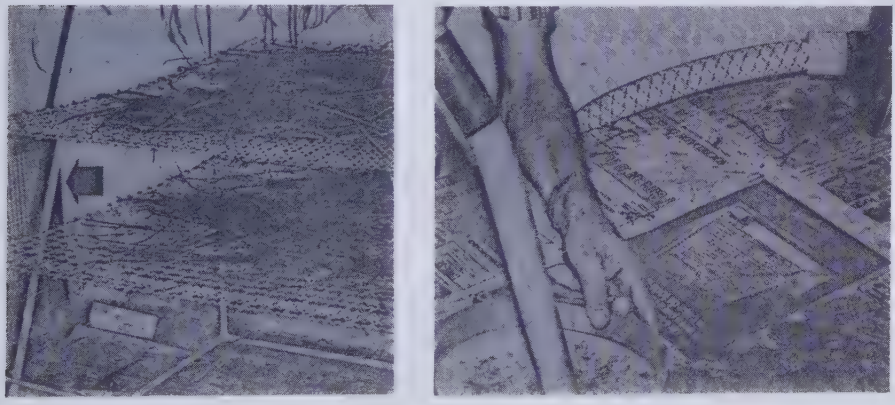
phases to evaluate the practical applicability of the method.

The following method has been used:

a) Whip harvesting in the field. The whips were cut in the field specially maintained as inoculum source, at the Plant Pathology Substation of PLANALSUCAR, located in Jacarei, State of Sao Paulo. Care was taken in order to harvest only apparently new ones. The cut was made at the base of the whip, and the excess leaves and leaf sheaths were removed. The whips were trimmed at the edge of the region where the spores were still covered by the silvery membranous sheath.

b) Whip preparation. After the whips were harvested they were taken to a nearby special room where the remaining leaf sheaths were removed. The youngest part of the whips, where the spores were not mature was also removed. A total lot of 469 whips was prepared and weighed. Care was taken during collecting and weighing to avoid the spores from failing on the table.

c) Whip and spore drying. After the whips had been prepared they were displayed on 20 cm apart shelves of wire screen, 2 x 2 cm, inside a 5 cubic-meter drying chamber containing an air dehumidifier (Fig. 1A). The fallen spores were also put in the chamber. The shelves and in providing good ventilation. The tempeature inside the chamber during this drying process varied from The temperature inside the chamber during this drying process varied from 24 to 30°C and the relative humidity of the air decreased from the initial 69.6% to 57.8% at the end of the drying process.



A B

FIGURE 1. Bulk harvesting method for smut spores

A. Inside view of the dehumidifying chamber showing spread smut whips on shelves and the electric dehumidifier (arrow).

B. Collecting spores dislodged from whips with vacuum cleaner.

d) Spore collection. A domestic vacuum cleaner, 1 HP, provided with a disposable paper bag collector, was utilized to collect the spores. The whips were lightly agitated on the shelves to release the spores still attached to them. The spores were then collected with the aid of the vacuum cleaner. A plastic sieve of 25 mesh was placed in front of the aspirator tube to eliminate large debris (Fig. 1B). Later on, the whips were placed on a table where they were again agitated, releasing more spores that were collected by aspiration.

After this operation, spores, whip cores and debris were weighed separately.

e) Final spore processing. For spore processing, a 100 mesh sieve was adapted to a funnel attached to the aspirator tube (Fig. 2). The spores, placed on the sieve, were sucked through the funnel and collected in the disposable paper bag collector of the vacuum cleaner, previously weighed. To improve sieving efficiency the spores were constantly moved in the sieve with a soft brush. After processing, spores and impurities were weighed separately.

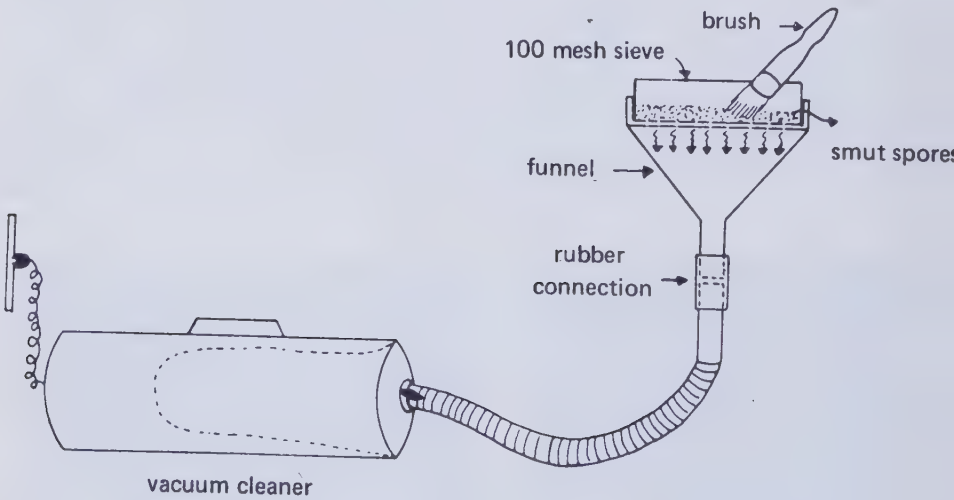


FIGURE 2. Set-up of smut spores sifting system

f) Drying and conservation of spores. In the laboratory, the spores were dried in a desiccator containing silica-gel. The spores were spread on a sheat of semipermeable paper inside the desiccator and the silica-gel substituted frequently. The drying was considered complete when spore weight and the blue color of silica-gel became constant. After this period, the spores were packed in small semipermeable paper bags, each bag containing *ca* grams of spores. These packages were placed into a desiccator, containing silica-gel, and stored in the refrigerator at 5°C[3,4].

Germination tests

Twelve lots of spores were collected from April, 1978 to March, 1979. The measurements herein described were collected from the twelfth lot. Lot nos. 1 and 2 were collected in March and April, 1978, according to the conventional method, whereas lots no. 3 to 12 were collected according to this newly-proposed method, from November, 1978 to March, 1979.

Every lot of spores, after the final drying, were tested for germination to evaluate the initial percentage of viable spores in the lot. Lot nos. 1 to 3, were germinated in distilled water, lot no. 4, in 2% sucrose solution and; the remaining lots, in water agar (20 g/1)[2,3,4].

A final germination test was carried out on March, 28, 1979, for all lots to evaluate the spore viability after different periods of storage. Germination tests were made on water agar.

RESULTS AND DISCUSSION

The spore collection using the vacuum cleaner was done 16 hours after the whips and spores had been placed in the drying chamber. The spore collection was done within 1 hour and the subsequent processing in 20 minutes. The final drying process took 5 days.

The weighing done during all these spore collection steps are presented in Table 1. It can be seen that the drying chamber efficiency is good, for, 67% of the moisture was eliminated from the initial gross material, thus avoiding the use of hard brush to dislodge spores from the whips.

TABLE 1. Quantities, (g) of spores and impurities obtained from 469 whip samples of *Ustilago scitaminea* artificially dried and collected with a vacuum cleaner

Steps	Whips	Spores released	Debris	Total
1. Material ready for drying in the chamber	1,851.0	89.0*	——	1,940.0
2. Material after drying	495.0	139.0**	18.0	652.0
3. After processing in 100 mesh sieve	——	124.7	11.1	135.8
4. After final drying in desiccator	——	112.5	——	112.5

**Spores that fell on the table during preparation of whips for drying.*
***Spores obtained from the whips, including spores of step 1, after drying in the chamber.*

After passing the spores in the 100 mesh sieve, 124.7 g of spores and 11.1 g of impurities were obtained, indicating a loss of 3.2 g of spores during

the process. This loss is due to the fact that many spores adhered to the paper bag and to the vacuum cleaner tube, while the other part went through the collector bag and were thrown away with the air expelled by the equipment.

It was observed that the final drying in the desiccator was necessary because the spores partially dried in the chamber still had about 9% of moisture removable by silica-gel.

TABLE 2. Germination tests of different lots of *Ustilago scitaminea* spores artificially dried, collected with a vacuum cleaner and stored at 5°C in desiccator containing silica-gel.

Lot[a] number	Spores (gram)	First test[b]		Second test	
		Substrate	Germination %	Months after the first test	Germination %
1	470.0	Water	74.0	11	40.6[c]
2	168.0	Water	75.0	10	60.1
3	108.0	Water	70.0	4	89.0
4	232.0	Sucrose[d]	89.0	4	87.5
5	184.3	Agar	93.0	3.5	89.0
6	231.8	Agar	93.0	3.5	89.0
7	117.0	Agar	93.0	3	88.0
8	482.0	Agar	91.0	3	88.0
9	539.0	Agar	88.7	2	86.0
10	279.0	Agar	86.0	2	78.0
11	484.0	Agar	90.0	0.5	90.0
12	112.5	Agar	88.0	0	88.0

a = Lot nos. 1 and 2 were naturally dried and processed without the use of vacuum cleaner.
b = The tests were carried out after final drying, within 2 weeks after the spores had been collected
c = All tests were carried out on water agar
d = A 2% sucrose solution

The data on germination tests are presented in Table 2. Germination in distilled water was the lowest which was probably due to the anaerobic conditions in the water. The spores did not germinate under a 5 mm film of water. This phenomenon was observed with lot no. 3 which showed a higher germination rate after 4 months of storage as compared to the initial rate. On the other hand, a decrease in germination rate was observed in lot nos. 1 and 2 which were collected following conventional method. However, it is not possible, based only on these results, to state that spores collected by the new method are better for storage, as the older spore collected by this method have been maintained only for 4 months. According to Mata[3], spores kept at 5°C in desiccator containing silica-gel, showed pronounced

decrease in viability after 12 months, whereas Leu[2] reported that under this condition, the spores maintained high viability up to 4 years. So, the viability of these lots should be followed up in further tests to accumulate sufficient data for a more critical conclusion.

CONCLUSIONS

The bulk harvesting method proposed has the following advantages: a) It accelerates the drying process, avoiding spore germination; b) Spore dispersion into the air is greatly reduced, thus eliminating the unpleasant aspects of collecting spores; c) The use of vacuum cleaner greatly facilitates spore collection and processing; d) Compared to the conventional method 60 to 70% of the total time required to perform all operations is reduced.

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UN METODO PARA COSECHAR LAS ESPORAS DE *USTILAGO SCITAMINEA* SYD. A GRANEL

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RESUMEN

Se describe un metodo que permite cosechar en grandes cantidades las esporas del carbon de la caña de azucar dentro de un corto periodo de tiempo:

(a) Se cosechan las esporas del carbon en el campo y se traen las esporas a un cuarto de procesamiento. (b) Se sacan las hojas y las vainas para exponer los latigos y secarlos completamente. La parte baja de los latigos, donde las esporas no estan maduras, tambien puede eliminarse. (c) Se esparciran los latigos sobre tablillas de cedazos

de alambre dentro de una camara desecadora pequeña equipada con un deshumidificador electrico. (d) Despues de 12-16 horas las esporas estan suficientemente secas para colectarlas con una limpiadora de vacio (1 Hp motor) provisto con una bolsa de papel desechable. (e) Se cerniran las esporas colectadas en un cedazo de malla No. 100. El cedazo esta ajustado a un embudo conectado al tubo de una aspiradora para facilitar el cernido. (f) Se almacenaran las esporas bajo 50°C en una desecadora conteniendo silica-gel. Este metodo reduce el tiempo requerido para elaborar las esporas considerablemente cuando se compara con el metodo convencional que se ha utilizado. Las pruebas de germinacion demonstraron que la viabilidad de las esporas colectadas por este metodo variaba desde 86% a 93%.

EFFECT OF VACUUM INOCULATION METHOD ON SUGARCANE CARYOPSES COLONIZATION BY *USTILAGO SCITAMINEA*

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ABSTRACT

When sugarcane caryopses are chlamydospores of *U. scitaminea* were submitted to vacuum pressures of 250 to 550 mm Hg, the germination of the chlamydospores was not affected while the caryopses at 250 mm Hg suffered 14% reduction in germination.

Caryopses inoculated with *U. scitaminea* by the vacuum method produced 36% colonized seedlings. When the same caryopses were inoculated without the vacuum treatment, the colonized seedlings dropped to 21.10%

Dehulled caryopses inoculated without vacuum treatment produced 42% of seedlings colonized which is similar to caryopses with hull inoculated with the vacuum method.

INTRODUCTION

One of the objectives of seeking varieties resistant to *Ustilago scitaminea* is obtaining an early screening method to decrease the breeding cost.

Tisdale and Tapke[12], and Bayles and Coffman[1], while working with corn and oat seeds found that when caryopses were inoculated without palea and lemma, there was more infection caused by *Ustilago* spp. Morre[7] and Shands and Schaller[9] using a partial vacuum method to inoculate corn and barley seeds with chlamydospores of *Ustilago* sp., obtained good results, although very variable. Kavanagh[4,5] also used various vacuum pressures to inoculate corn and burley seedlings with *Ustilago* sp., and reported that the chlamydospores concentration that produced the best results was 1 gram per liter of water and the seedlings were subjected to vacuum for 2 minutes.

Talballa[12] while working with sugarcane, observed that caryopses obtained from arrows contaminated by spores of *U. scitaminea* produced seedlings with whips 10 weeks after sowing, revealing that this fungus can cause diseases in seedlings.

Mata and Tokeshi[6] subjected chlamydospores of *U. scitaminea* to a vaccum pressure of 760 mm Hg for 2 minutes, and found that the chlamydosphore germination was not affected.

Duarte & Tokeshi[3] got high percentage of seedlings colonization by *U. scitaminea* when inoculating seedlings produced from caryopses without palea and lemma. This work was carried out under laboratory conditions to get a method of inoculation of sugarcane seedlings with *U. scitaminea* and to avoid problems caused by husk and the dehulling of caryopses found in previous work.

MATERIALS AND METHODS

During inoculum preparation, chlamydosphores of *Ustilago scitaminea* were processed and stored according to the method presented by Mata and Tokeshi[6]

With the use of hemacytometer, inoculation with a concentration of 6×10^6 spores/ml was prepared from chlamydospores with 81% of germination.

Caryopses of the variety RB 68121 were pre-cleaned by Silva[10] method and cleaned with seed blower regulated at 9.6 mg/cm^2, according to Bleicher and Tokeshi[2]. With the objective of disinfesting caryopses, plots of 400 mg were treated with zinc-2-pyredimethiol-l-oxide (zinc ornadine) at the concentration of 1440 ppm for 2 hours and rinsed with distilled water.

The test consisted of 5 treatments, 4 replications and each plot had 100 caryopses.

Each treatment was placed in Erlenmeyer bottles with 200 ml of distilled water and submitted to different vacuum pressures for 2 minutes. Caryopses were then sowed in trays with water agar medium and incubated under 40 watts of fluorescent lamps, at a distance of 90 cm from the light source, for seven days at 29°C. After this period the germinated caryopses were counted.

In the test using CO 62-175, the caryopses were cleaned with seed blower as described above. The seeds were divided in lots, each lot received one of the following treatment: a) caryopses dehulled were immersed in a diluted solution of commercial sodium hypochlorite (Q-BOA) with 5.5% of active chlorine diluted in a proportion of 1:3 v/v for 10 minutes, as described by Duarte and Tokeshi[3]. After disinfection, caryopses were inoculated; b) caryopses with husks (palea and lemma) were immersed in a diluted formaldehyde (Lysoform bruto), with a proportion of 1:20 v/v for 20 minutes, rinsed with water and then inoculated with spore suspension under a vacuum pressure of 250 mm Hg for two minutes; c) caryopses with husk were immersed in diluted formaldehyde at a rate of 1:20 v/v for 20 minutes, rinsed with water and then inoculated by immersion for two minutes without vacuum.

Inoculation by immersion was carried out upon sowing and by spraying on the 2nd and 8th day with an inoculum identical to that used in previous test. Three days after sowing, samples of 30 seedlings were transplanted to a 25 ml water agar medium. Twelve days after sowing seedlings were gathered and stained, using the Popp method[8], and the seedlings examined under the microscope to determine the internal presence of *U. scitaminea* mycelium, according to Duarte and Tokeshi[3]. The results were transformed into percentage of colonized seedlings. For statistical analysis, this data was transformed into arc sin $\sqrt{\bar{X}}$. The test consisted of 3 treatments, 12 replications and each plots containing 30 seedlings.

RESULTS AND DISCUSSION

Vacuum pressures between 250 to 550 mm Hg do not affect the chlamydospores germination (Table 1). Mata and Tokeshi[6] also showed that chlamydospores did not suffer any effect on their germination when submitted to a vacuum pressure of 760 mm Hg.

TABLE 1. Vacuum effect on percentage germination of 400 chlamydospores of *Ustilago scitaminea*

Vacuum mm Hg	Replications			Mean	Transformed Mean
	1	2	2		
0	78.48	75.40	77.00	76.96	61.30a*
250	78.40	76.50	75.44	76.11	60.73a
350	77.05	76.78	73.34	75.72	60.48a
450	76.21	77.49	77.23	76.97	61.32a
550	74.70	76.83	71.59	74.37	59.60a

C.V. = 1.89 M.S.D. 5% = 3.00

**Mean with the same letter are statistically the same at 5% level through Tukey test.*

Caryopses of sugarcane submitted to the same vacuum pressures suffered a reduction on germination when compared to those which were not submitted to the vacuum (Table 2). Statistically, there were no significant variations in the mean percentage among the different vacuum pressures used. The decrease in percentage of germination in the vacuum inoculation method are probably due to the mechanical damage caused in weaker embryos.

When the vacuum was used in sugarcane caryopses inoculation with *U. scitaminea,* there was an increase in percentage of colonized seedlings (Table 3). Seedling from caryopses inoculated by vacuum method produced 42% more infected plants than from those caryopses not subjected to the vacuum inoculation method.

TABLE 2. Effect of vacuum method on % germination of 100 sugarcane caryopses, Variety RB 6812

Vacuum mm Hg	Replications				Mean	Transformed Mean
	1	2	3	4		
0	831	75	84	82	81.00	64.24a*
250	61	62	74	68	64.25	54.54b
350	62	68	73	64	66.75	54.82b
450	65	65	58	63	62.75	52.39b
550	63	66	66	59	63.50	52.84b

C.V. = 9.12% M.S.D. 5% = 7.72

**Means with the same letter are statistically the same at the 5% level through Tukey test.*

TABLE 3. Effect of husks and vacuum inoculation method on sugarcane seedlings, Variety Co 62-175, colonization by *Ustilago scitaminea*

Plots of 30 seedlings	Percentage of colonized seedlings produced from inoculated caryopses		
	No husks No vacuum	With husks No vacuum	With husks With vacuum
1	35.00	26.66	33.33
2	50.00	13.33	36.66
3	45.00	20.00	50.00
4	25.00	6.66	30.00
5	45.00	16.66	46.66
6	40.00	16.66	30.00
7	26.66	26.66	36.66
8	46.66	20.00	36.66
9	30.00	26.66	33.33
10	33.33	23.33	33.33
11	26.66	26.66	40.00
12	43.33	30.00	36.66
Mean	37.22	21.10	36.94
Transformed mean	37.47a*	27.02b	36.90a

C.V. = 19.67 M.S.D. 5% = 4.68

**Means with the same letter are statistically the same at the 5% level through Tukey test.*

Vacuum pressures might increase the percentage of colonized seedling by *U. scitaminea*, because chlamydospores are placed close to the embryo. Reports of studies in various species of grasses using vacuum inoculation method produced favorable results Morre [7], Shando and Schaller [9], and Kavanagh [4, 5]

Results show that the husk really interferes with the inoculation efficiency and comparable to those described by Tisdale and Tapke[12] and by Bayles and Coffman[1], in corn and oat. These results validate the hypothesis raided by Duarte and Tokeshi[3], that husk interfere in the colonization of sugarcane by *U. scitaminea.*

Statistically, the dehulled caryopses inoculated without vacuum is similar to caryopses inoculated with husks and under vacuum. This demonstrate that the method described by Duarte and Tokeshi[3] is equivalent in efficiency to the method described in this study. The major advantage of the use of vacuum in inoculations is the elimination of dehulling operation to decrease seed contaminants interference in the screening of resistant plants.

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EL EFECTO DEL METODO DE INOCULACION VACIO EN LA COLONIZACION DEL CARIOPSES DE LA CAÑA DE AZUCAR POR EL HONGO *USTILAGO SCITAMINEA*

Jorge Bleicher and Hasime Tokeshi

RESUMEN

Cuando cariopses y clamidoesporas del hongo *scitaminea* fueron sometidos a una presion de 250-550 mm Hg, se encontro que las clamidoesporas no fueron afectadas en su germinacion, mientras los cariopses sufrieron un 14 porciento de reduccion en la germinacion.

Cariopses inoculados con el hongo *U. scitaminea* por el metodo de vacio produjeron un 36 porciento de plantas de semillas colonizadas. Cuando los mismos cariopses fueron inoculados sin tratamiento de vacio, el porcentage de semillas colonizadas se redujo al 21.10%.

Cariopses descascarados e inoculados sin tratamiento de vacio produjeron una frecuencia de semillas colonizadas semejantes al cariopses con cascara e inoculadas con el metodo de vacio. Ambos tratamientos presentaron una eficiencia del 42% sobre la inoculacion de cariopsis con cascara y sin vacio.

INFECTION OF THE BUD, ESTABLISHMENT IN THE HOST AND PRODUCTION OF WHIPS IN SUGARCANE SMUT *(USTILAGO SCITAMINEA* SYD.) OF SUGARCANE

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ABSTRACT

Entry into the meristem in the bud occurred between 6 hours and 36 hours after the teliospores got deposited on the bud. The infection hyphae did not penetrate and colonize the cells of the scale leaves. However, they were found to circumvent to reach the base of the innermost scale leaf and to affect an entry into the meristem at this region. Hyphae were noticed throughout the infected plant mostly in the parenchyma cells towards the lower internodes. In the upper internodes, the hyphae were progressively built up culminating in the formation of sori with teliospores.

INTRODUCTION

The parasitic phase in *Ustilago scitaminea* Sydow is initiated with the germination of the teliospores which lodge on the bud. However, successful infection takes place only after the infected hyphae attain the dikaryotic condition.

MATERIALS AND METHOD

Buds of Co 213 were smeared with teliospore suspension and incubated at 30°C. The buds were fixed in at intervals of 6, 12, 18, 24, 36, 48 and 72 hours. Longitudinal sections were cut with a hand microtome and the sections were differentially stained with haematoxylin and safranine and examined under the microscope.

Progress of infection in the host

Single bud setts were prepared from a whip formed cane of Co 213. To ensure infection, these were again stepped in a spore suspension and buds were planted in microplots. At about 60 days after planting, some seedlings with obvious symptoms of infection like erectness of leaf and thick culm, were removed. One such infected seedling with the whip still within the spindle was removed and separated from the sett. The leaf with the sheath was removed and the plant was cut and numbered serially, photographed and fixed with FAA (Fig. 1). The fixed

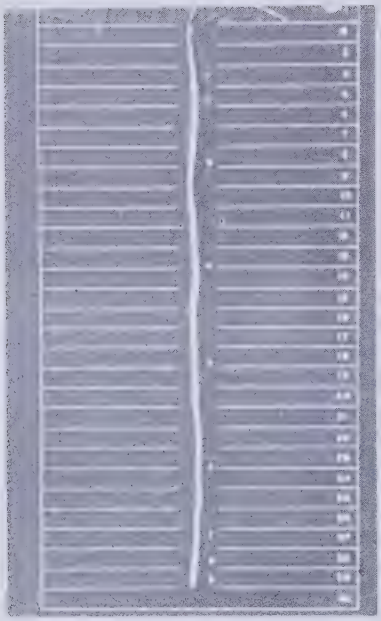

FIGURE 1. Infected plant prepared for sectioning

material was processed for microtomy and serial sections were cut with thickness of 12 μ. The sections were stained in haematoxylin for 2 hours and then counter-stained in safranine for 30 minutes. It was found that all the lignified and thick walled cells were stained spinel pink, the thin walled cells were stained manganese violet and the fungus hyphae were stained nopal red.

RESULTS

Pathway of infection in bud

Six hours after inoculation the teliospores had germinated and initiation of the dikaryotic condition of the infection hyphae had occurred. After 12, 18, 24, and 36 hours, progress of infection was noticed. The infected hyphae had proliferated along the outer scales of the bud and continued along the inner edge of the outer scales. The hyphae were stained nopal red and the meristem cells, manganese violet. Such elongated hyphae were also noticed on the inner and outer surfaces of the inner scale leaves. In buds where the scale leaves were not closely packed and the inner scale leaves were exposed, hyphae were found at the base of the innermost scale leaf which immediately covered the bud. The hyphae had not penetrated the bud scales but were growing along the outer and inner surfaces of the scale leaves which covered the bud. The hyphae then enter the meristem region and the surrounding tissue through the basal region of the innermost scale leaf. In cross sections, the hyphae were found to be inter-cellular with small knob-like inconspicuous haustoria. Entry into the meristem occurred between the 6th and 36th hour after inoculation of the bud in the variety Co 213.

Progress of infection in the host:

The infected plant was divided lengthwise into 29 equal bits and the entire stem was serially sectioned beginning from the bottom, i.e. the 29th sector onwards to the number one sector. After differentially staining the sections in haematoxylin and safranine, they were examined under the microscope.

Sector 29 is comprised of the lowermost 9th internode and the bottom of the 8th internode. The sections when examined serially, showed mostly intercellular fungal hyphae. When intracellular fungal hyphae were shown they appear as short knobs or slightly lobed and stained nopal red. Fungal hyphae were observed only in parenchyma cells, adjacent to vascular bundles. Sometimes they were also seen away from the vascular bundles. Fungus hyphae were observed only in very few cells. From the 29th to the 20th sector, comprised of the 8th, 7th and 6th internodes, infection was similar to what was observed earlier.

In the fifth internode (sector 19), sections when examined showed the fungal hyphae putting forth initial branches and were also slightly elongated. However, the fungus was found only in the parenchyma tissue. The fungus was also observed more frequently permeating the tissue, though still observed in or adjacent to old, well separated cells.

In the 3rd and 4th internodes (sectors 11 to 9), hyphae were branched and elongated in parenchyma cells. They were found as short knobs for the first time within phloem cells. In the 3rd and 2nd internodes (sectors 8 to 50, thickening of the hyphae were seen in the parenchyma cells just below the epidermis. Host cell walls have collapsed and hyphae were accumulated in irregular sori initials. Epidermis was intact and continuous. In the second and first internodes, sections were seen with enlarged sori initials. They coalesced with the ones below. Hyphae within the sori were rounded, thick and brown in color. In the first internode below the whip, there was no external symptom of infection. Sections showed rounded thick hyphae forming immature, intercalary teliospores. In further sections, sori were enlarged with closely packed maturing teliospores. In still further sections, there were no evidence of any host tissue. Sori were enclosed in a single layer of thin walled cells which formed the solar or silvery membrane.

DISCUSSIONS AND CONCLUSION

Entry into the meristem was attained between 6 and 36 hours after the teliospores got deposited on the bud. The infected hyphae have not been seen to penetrate and colonize the cells of the scale leaves but were circumventing them to reach the base of the innermost scale leaf. They affected an entry at this region into meristem. Hence, buds which are completely and tightly enclosed with scale leaves have a better way of escaping infection than buds which are not. It has also been observed that removal of budscales covering the bud before inoculation, leads to better infections (Singh and Budhraja[1], Waller[2]).

From the observations made on the progress of infection in the host, it was evident that the fungus was present throughout the plant. As the infection progressed, the fungus became more conspicuous in the parenchyma cells and later also in the phloem cells. The sori initials were formed far below the region from where the whip emerged and were progressively built up to form sori with teliospores. In the lower parts of the plant, the host cell is apparently unaffected and cell disorganization is noticed only much later in the infection stage, as the hyphae accumulate within the cells. Fawcett[3] reported that the mycelium was never present in the internode or elsewhere. He also considered that each diseased bud represented an independent and separate unit of infection. Leu[4] found hyphae 5 to 6 cm below the point from which the whiplike structure originated. The present study showed that hyphae is present throughout an infected plant.

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LA INFECCION DE LA YEMA, ESTABLECIMIENTO EN EL HUESPED Y PRODUCCION DE LATIGOS DEL HONGO CAUSANTE DEL CARBON *(USTILAGO SCITAMINEA)* EN LA CAÑA DE AZUCAR

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RESUMEN

La penetracion del hongo en el meristem de la yema ocurrio unas 6 a 36 horas despues de que las telioesporas se depositaron en la yema. Las hifas de infeccion no penetraron ni colonizaron las celulas de las hojas iniciales, pero se encontró que las rodeaba hasta llegar a la base de las hojas iniciales mas internas. Se llevo a cabo una penetracion al meristem en esta region. Se encontraron hifas en la planta infectada, mayormente en las celulas de parenquima hacia los entrenudos inferiores. En los entrenudos superiores, las hifas se acumularon progresivamente, culminando en la formacion de soros con telioesporas.

REACTION OF SUGARCANE CLONES TO STRAIN B OF *USTILAGO SCITAMINEA* SYD.

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ABSTRACT

Among the Hawaiian clones tested H 70-2665, 71-4919 and 66-3732 were found to be very highly resistant. H66-2182 and 70-1092 were highly resistant, H 68-7482 and H 70-9455, were resistant and intermediate resistant, respectively.

Seven clones of the 1974 series, 74 P 1693, 1505, 1375, 0347, 2747, 0545 and 0305 were rated very highly resistant. Three clones, 74 P 1293, 2885 and 0549 were rated highly resistant and one clone 74 P 1501, was rated resistant. Only one clone 74 P 2721 was rated intermediate resistant.

In the 1975 series, three clones, 75 P 1121, 0545 and 0607 were found very highly resistant. Seven clones, 75 P 0327, 0155, 0371, 0417, 1695, 0293 and 0369 were found highly resistant. Nine clones, 75 P 0351, 0279, 0539, 0765, 0289, 0667, 0309, 0825 and 0047 were found to be resistant.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of the major crops as well as one of the dollar earning crops of the Philippines. It is estensively grown in many areas in Negros, Panay, Leyte, Cotabato, Davao, Batangas, Laguna and Pampanga.

Like other crops, sugarcane is attacked by different diseases most notable of which is smut (*Ustilago scitaminea*). Attempts to control the disease by the use of resistant varieties and hot water treatment of seedpieces have been reported (Thompson[8]). However, the use of resistant varieties is more promising. Severe outbreaks of the disease have been checked in many countries through the use of resistant varieties (Antoine[1]).

Testing of varieties against smut on a large scale is being undertaken at Philsucom, La Granja Sugarcane Research Station.

Rivera[5] reported varieties CP 29/116 and NCo 310 to be highly resistant to

smut; POJ 3016 as average, B 37/122 as highly susceptible; and H 44-3098 as very highly susceptible Rivera *et al*[6] found that out of the 43 Phil. varieties tested to smut, 6 were found very highly resistant, 18 were highly resistant, 10 were moderately resistant, 6 were average and 3 were moderately susceptible. They also reported that 4 oustanding Phil. commercial varieties were rated as follows: Phil. 5333, highly resistant; 5460, 56226 and 58260, moderately resistant. These varietal reactions were found to be relatively similar to those occurring under commercial field conditions. Inspite of the availability of information on the resistant or susceptibility of the cane varieties in the Philippines to smut, no work has been done regarding the use of identified strains as source of inoculum for screening, hence this study.

Since smut is a disease of economic importance, all promising varieties produced by the Breeding Department undergo routine smut disease resistance trials. This paper presents the reaction to smut of sixty one Hawaiian clones, thirty three clones of the 1974 series, and forty seven clones of the 1975 series. The experiments were conducted from January, 1977 to December, 1978 at the Department of Crop Protection, Philsucom, La Granja Experiment Station, La Carlota City.

MATERIALS AND METHODS

The susceptibility or resistance to smut of 141 clones of sugarcane was tested. Sixty one clones that came from Hawaii were tested as part of the cooperative study of Philsucom-Hawaii. Thirty three clones of the 1974 series and 47 clones of the 1975 series were included in the study.

TABLE 1. Smut Rating Scale

Reaction	Smut grade	Stools infected (%)
Very highly resistant	1	1.0 – 2.5
Highly resistant	2	2.6 – 5.5
Resistant	3	5.6 – 7.5
Intermediate resistant	4	7.6 – 12.5
Intermediate average	5	12.6 – 15.5
Intermediate susceptible	6	15.6 – 18.0
Susceptible	7	18.1 – 22.5
Highly susceptible	8	22.6 – 22.5
Very highly susceptible	9	25.6 – 100

Source of inoculum. The inoculum used in the experiment was Strain B of the fungus which was collected from Isabela, Negros Occidental.

Method of inoculuation. The dipping method of inoculation was used. This was done by dipping cane points for 10 min. in spore suspensions (5×10^6 spores/ml).

Collection of data. The occurrence of smut whips was observed and recorded at monthly intervals until the canes were eight months old. The degree of infection of the canes were expressed in percent of infected stools.

A stool was considered infected when smut appeared on one or more stalks arising from one cane point. The results were finally expressed in total percent infected stools. Percentage infection was computed by adding the number of infected stools divided by the total shoots in the row, then multiplied by 100. The average of the replicate (10 stools per replicate) represented the reaction of a certain clone. The smut reaction was graded on a 1 to 9 scale as indicated in Table 1, grade 1 being the most resistant and grade 9 the most susceptible.

RESULTS AND DISCUSSIONS

The results of the experiments are shown in Tables 2, 3 and 4. The reaction of clones varied from very highly resistant to very highly susceptible. Among the Hawaiian clones tested, three clones were found to be very highly resistant, H 70-2665, 71-4919 and 66-3732; two clones, H 66-2182 and 70-1092 were highly resistant; two clones, H 68-7482 and H 70-9455 were resistant and intermediate resistant, respectively. The other clones were found to be intermediate susceptible to very highly susceptible.

TABLE 2. Reaction of Hawaiian clones to Strain B of *U. scitaminea*

Clones[a]	Rating	Reactions
H 70-2665	1	Very highly resistant
H 71-4919	1	Very highly resistant
H 66-3732	1	Very highly resistant
H 66-2182	2	Highly resistant
H 70-1092	2	Highly resistant
H 68-7468	3	Resistant
H 70-9455	4	Intermediate resistant

[a]*Conducted during the year 1977-78.*

TABLE 3. Reaction of 1974 Phil Clones to strain B of *U. scitaminea*

Clones[a]	Rating	Reactions
74 P 1693	1	Very highly resistant
74 P 1505	1	Very highly resistant
74 P 1375	1	Very highly resistant
74 P 0347	1	Very highly resistant
74 P 2747	1	Very highly resistant
74 P 0545	1	Very highly resistant
74 P 0305	1	Very highly resistant
74 P 1293	2	Highly resistant
74 P 2885	2	Highly resistant
74 P 0549	2	Highly resistant
74 P 1501	3	Resistant
74 P 2721	4	Intermediate resistant

[a]*Conducted during the year 1977-78.*

In the 1974 series (Table 3), 7 clones, 74 P 1693, 1505, 1375, 0347, 2747, 0545 and 0305 were rated very highly resistant. Three clone viz., 74 P 1293, 2885, and 0549 were rated highly resistant and 1 clone 74 P 1501, was rated resistant. Only 1 clone 74 P 2721 was rated to be intermediate resistant. Twenty-one clones were rated intermediate susceptible to very highly susceptible.

Table 4 shows the reaction of the 1975 clones to smut. Out of 47 clones tested, 3 clones viz., 75 P 1121, 0545 and 0607 were found very highly resistant. Seven clones, viz., 75 P 0327, 0155, 0371, 0417, 1695, 0293 and 0369 were found highly resistant. Nine clones, viz., 75 P 0351, 0279, 0539, 0765, 0289, 0667, 0309, 0825 and 0047 were found to be resistant.

It was observed that resistant clones exhibited smut infection at 4 to 5 months while the intermediate resistant and the susceptible clones at about 3 months and 1-1/2 months, respectively. The delay in the expression of symptoms in the resistant varieties may be due to chemical, morphological, physiological or genetic factors. Chemicals in plants play a role in protecting them against plant pathogenic microorganisms. Kuo[4] reported that phenolic compounds make plant resistant to certain disease. High reducing sugars and moisture and low fiber were reported correlated with susceptibility of sugarcane varieties to downy mildew (Stevenson[7]). However, Fawcett[2] demonstrated that immunity of varieties to smut infection under normal conditions was due to physical properties of the bud and

TABLE 4. Reaction of 75 Phil clones to Strain B of *U. scitaminea*

Clones[a]	Rating	Reactions
75 P 1121	1	Very highly resistant
75 P 0545	1	Very highly resistant
75 P 0607	1	Very highly resistant
75 P 0327	2	Highly resistant
75 P 0155	2	Highly resistant
75 P 0371	2	Highly resistant
75 P 0417	2	Highly resistant
75 P 1695	2	Highly resistant
75 P 0293	2	Highly resistant
75 P 0369	2	Highly resistant
75 P 0351	3	Resistant
75 P 0279	3	Resistant
75 P 0539	3	Resistant
75 P 0765	3	Resistant
75 P 0289	3	Resistant
75 P 0667	3	Resistant
75 P 0309	3	Resistant
75 P 0825	3	Resistant
75 P 0047	3	Resistant

[a]*Conducted during the year 1977-78.*

not due to any chemical substances present in the composition of the plant. Likewise, Leu[3] showed that the number of chromosomes in the clones of *Saccharum spontaneum* has no correlation with their susceptibility.

SUMMARY

One hundred forty-four clones were screened for resistance to strain B of sugarcane smut during the years 1977 to 1978 at the Philsucom; La Granja Sugarcane Experiment Station. Sixty-one clones came from Hawaii, thirty-three clones of the 1974 series and 47 clones of the 1975 series were included in the study. Among the clones tested, 13 were classified as very highly resistant, 12 highly resistant, 11 resistant and two intermediate resistant.

The susceptible clones exhibited smut symptom much earlier than the resistant clones.

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REACCION DE LOS CLONES DE LA CAÑA DE AZUCAR AL LINAJE B DEL *USTILAGO SCITAMINEA* SYD

A.S. Latiza, D.C. Ampusta y J.R. Rivera

RESUMEN

Entre los clones Hawaiianos probados, tres clones fueron encontrados altamente resistentes. Estos fueron: H 70-2665, 71-4919 y 66-3732; dos clones, H 66-2182 y 70-1092 fueron altamente resistentes; dos clones, H 68-7482 y H 70-9455 fueron resistentes y moderadamente resistentes respectivamente.

En las series de 1974, siete clones viz., 74 P 1693, 1505, 1375, 0347, 2747, 0545 y 0305 fueron clasificadas muy altamente resistentes. Tres clones, viz., 74 P 1293, 2885 y 0549 fueron clasificados altamente resistentes y un clone, 74 P 1501 fue clasificado resistente. Solamente

un clone, 74 P 2721 fue clasificado moderadamente resistente.

En las series de 1975, tres clones 75 P 1121, 0545,y 0607 fueron encontrados muy altamente resistentes. Siete clones, 75 P 0327, 0155, 0371, 0417, 1695, 0293 y 0369 fueron encontrados altamente resistentes. Nueve clones, 75 P 0351, 0279, 0539, 0765, 0289, 0667, 0309, 0825 y 0047 fueron encontrados resistentes.

EVALUATING SUGARCANE SMUT RESISTANCE

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ABSTRACT

Since the use of resistance is the most important control strategy for sugarcane smut (*Ustilago scitaminea* Sydow), procedures for inoculation and evaluation of varietal resistance in different countries are reviewed. Data are also presented suggesting that smut races may exist generally and that, in Hawaii, inheritance of resistance to race A is independent of that to race B. Recommendations are made for standardizing certain smut testing procedures and for reporting varietal smut reactions. This would help answer the question of the extent to which smut races exist and may provide data on smut race dynamics.

INTRODUCTION

Culmicolous smut (*Ustilago scitaminea* Sydow) of sugarcane is cosmopolitan in distribution and at one time or another has proved to be significant in nearly all the sugarcane-producing countries of the world. Originally reported in Natal in 1877, smut was confined to the eastern hemisphere until it was discovered in the Tucuman of Argentina in 1940 (Antoine[2], Cross[7], Lee-Lovick[20], Presley[27], Sugarcane Smut Workshop[37]). Since that period, the disease has become widespread in both hemispheres, being most recently reported in the last 5 years in Guyana, Jamaica, Trinidad, Martinique, Belize, Venezuela, Cuba, St. Kitts, Honduras, and Florida (Presley[27], Sugarcane Smut and Rust Workshop[37]). At this writing only Australia and Fiji remain free of this disease.

Sugarcane smut has been relatively successfully controlled. As is the case with other smut diseases, a control program has evolved using the principles of sanitation (roguing of stools and/or whips), clean seed (hot-water treatment of seed setts), and resistance. Although the control program is an integrated one, its most important element has been the reliance on resistance. One purpose of this paper is to review and assess the effectiveness of various techniques and procedures employed in the sugar industry to evaluate varieties for smut resistance.

When the disease first appears in a country, its history is usually one of severe epidemics followed by a period of relative unimportance. Thereafter, a cycle may be established of severe and mild disease outbreaks (Antoine[2]). Sometimes, however, after its initial epidemic period, smut never proves to be important again. The cause for these different behavior patterns is not known. It is possible that environment-pathogen interaction might be a partial explanation. It is possible also that races of the pathogen exist and are responsible for the disease patterns observed. However, races have been reported only from Hawaii, Taiwan, Brazil, and Pakistan (Comstock and Heinz[5], da Silva and Sanguino[8], Hsieh and Lee[13], Lee-Lovick[20], Leu and Teng[21], Muhammad and Kausar[26], Toffano[38]).

It is important that sugarcane smut races and their dynamics be more completely understood. The existence of races has consequences, bearing on variety development programs as well as on the successful use of resistance in smut control. If new and more virulent races are evolving, the effective and long-term use of resistance may be impaired. New sources of resistance may be required and will have to be incorporated into promising varieties. We shall review the evidence concerning the existence of smut races and suggest a methodology which should help to answer this question.

EXPERIMENTAL PROCEDURE

SMUT DISEASE EVALUATION METHODS AND SMUT RACES

Inoculation

In general, three inoculation techniques are employed in evaluating varietal reactions to smut. The most widely-used technique involves immersing seed pieces, generally one-eye or three-eye, in a teliospore suspension. Immersion time varies from 10 minutes to 12 hours and inoculum concentration from approximately 2.5×10^6 to 5×10^8 teliospores/ml. Sometimes inoculated seedpieces are subjected to an incubation treatment prior to planting. The various inoculation procedures among countries regularly screening for smut resistance are summarized in Table 1.

Another inoculation procedure, which relies on natural infection, is used in Rhodesia and Hawaii. Inoculated susceptible varieties are planted as spreader rows where inoculum is built up; test varieties are planted adjacent to spreader rows. Varieties are naturally infected although inoculum levels are usually higher than those observed in smutted commercial fields, especially when tests are ratooned several times. Generally, at least two ratoon cycles are required before a variety can be reliably assessed. The natural infection procedures for Rhodesia and Hawaii are given in Table 2.

A final inoculation variation is the wound-paste technique. It is the technique most commonly used in Taiwan, and it is used in conjunction with the immersion technique in Brazil. The procedure involves the use of two

TABLE 1. Summary of immersion inoculation methods in several countries

Country	Inoculum concentration (teliospores/ml)	Immersion time (minutes)	Test size (No. buds)	Preplant treatment	Age of crop at disease evaluation (months)	No. of crops	References
India	$1x10^6$ - $5x10^8$	30-45	60-100	Variable; 0-4 days in humidity tent	6-7	plant	3, 9, 16, 25, 30, 32, 33, 34, 35, 36
Taiwan	$1x10^7$	30	50	24 hours in plastic bags	7-9	plant, ratoon	21, 22, 23
Brazil	1 - $2x10^7$	10-20	20-40	Pregerminated in sand	3-6	plant, ratoon	1, 8
Philippines	$1x10^5$ - $1x10^6$	10-720	100	None	7-9	plant	11, 14, 19, 24, 28
Hawaii	$5x10^6$	10	45	Held overnight before planting	5-6	plant, ratoon	4

TABLE 2. Summary of natural infection methods in Hawaii and Rhodesia

Country	Plot layout	Plot size	Age of crop at disease evaluation (months)	No. of Ratoons	References
Hawaii	Test plots adjacent to spreader plots; double spreader line for border	15 seedpieces in 5 m plots, 4 replications	5-6	2	18
Rhodesia	Every third line a spreader line of NCo 376	12 m plots,	8-10	4-7	17

to six puncture wounds on or near the bud to enable the pathogen to gain access to the host after a thick paste of teliospores is brushed on the wounded bud. This technique is probably the best method to use when inoculum is not abundant. It is, however, one of the most unnatural and severe inoculation procedures available. Most workers report that this form of testing probably gives a severe estimate of disease reaction underestimating acceptable resistance (Bythers and Steiner[4], Lee-Lovick[20]).

Although we are not aware of much data having been accumulated to adequately evaluate the technique, another inoculation method used only in Kenya (Early[10], Waller[39]) employs an aerograph pencil or air brush spray with a force of 5.6 kg/cm^2. An aliquot of inoculum suspension of 1 x 10^6 teliospores/ml is directed at a bud from a distance of 1 cm with bud apices oriented toward the inoculum stream. Seedpieces are planted and disease evaluated at the usual time periods. An even more severe technique has been employed occasionally in Brazil. Seedpieces immersed in spore suspensions are subjected to partial vacuum pressures for up to 20 minutes. Levels of infection are considerably higher than with the immersion inoculation technique, but there is some injury to the lateral buds (Hirschhorn[12], Sanguino and Tokeshi[31], Singh *et al*[34], Toffano[38]).

Most countries generally rely on only one of the inoculation procedures described. In Hawaii and Brazil, two techniques are used to complement each other. In Brazil, all varieties are now screened by inoculating the first of two sets of seedpieces by the immersion technique and the second by the wound-paste technique. Results are interpreted in terms of maximal disease response. In Hawaii, primary reliance is placed on the immersion technique; only varieties with intermediate smut reactions, 4, 5 or 6 in the Hawaiian

rating scheme, and promising near-commercial varieties are evaluated further by natural infection testing.

With many years of testing using both methods, Hawaiian workers have shown a good correlation between results obtained from natural infection testing and immersion inoculation testing. Results shown in Fig. 1 were obtained in 1977. The correlation between natural and artificial infection was 0.7. In general, the immersion procedure tended to underestimate resistance. There were a few instances where higher disease ratings were obtained with the natural infection procedures.

The choice of inoculation procedure depends on several factors. The size of the testing program and availability of inoculum are the most important

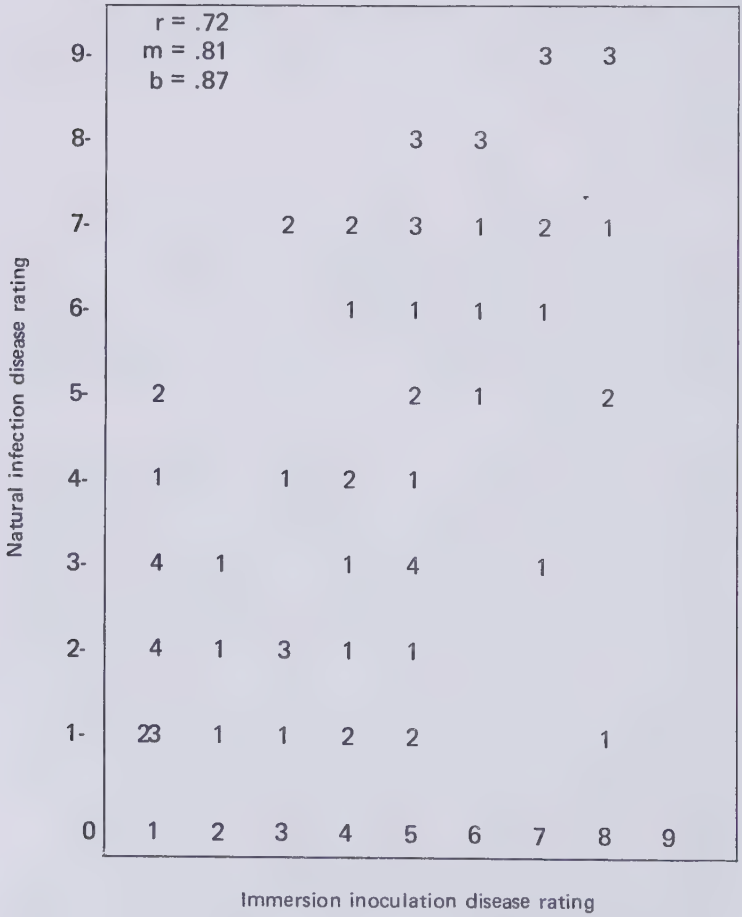

FIGURE 1. Comparison of disease ratings of 45 clones to smut races A and B from natural infection and immersion inoculation test in Hawaii. The numerals on the graph indicate the number of clones rated as indicated.

considerations. In addition, the time required to accumulate data determine the choice of method. It is difficult at this time to make a judgment as to the best inoculation procedure to use. More study is needed to understand *U. scitaminea,* its host-pathogen interaction, and the nature of resistance before a more critical analysis of procedures can be made.

Disease reaction evaluation

There is more agreement among workers on how to evaluate varietal smut reactions than on how to inoculate with the pathogen. All countries but Rhodesia rely on percent infection (stools or plants). In Rhodesia, an estimate of infection is made in small plots and data are extrapolated to the field basis. Infection is indicated as the number of smut whips observed per hectare.

All countries employ the 0-9 disease scale of Hutchinson[15] but differ in their assignment of infection percentage to disease rating. The resistant reaction ranges from 0 to 15%, with most workers judging 10% to be the dividing line between resistance and an intermediate reaction. For susceptibility, the dividing line ranges from 16% to 40% with a mode of about 26%. If the Rhodesian data were converted to a percent infection basis, assuming 75,000 stalks per hectare (15,000 stools per hectare), their break between resistance and susceptibility would occur at about 12.5%. Based on the Hawaiian and Rhodesian data there appears to be good agreement between the immersion

TABLE 3. Annual comparison of smut resistant hybrid seedlings produced in the Hawaiian variety development program since 1971

Year	Percentage seedlings resistant to race A
1971	25
1972	–
1973	55
1974	76
1975	83
1976	80[a]
1977	70
1978	71
1979	75

[a] *A second race of smut, race B, was discovered in 1976 where seedlings were first evaluated for smut resistance. The influence of this second race was probably responsible for the decline in hybrid seedling resistance since 1976.*

and natural infection methods in distinguishing resistant or susceptible host reactions.

In establishing guidelines for evaluating disease reactions, there is a constant debate among pathologists and breeders in distinguishing between resistance and susceptibility. The disease evaluation scheme developed in Hawaii appears about average or slightly less stringent than that used in most countries. Nevertheless, let us review the progress made in Hawaii with respect to developing smut resistant varieties. When smut was first discovered in Hawaii in 1971, for smut resistance was imposed, considerable progress was made in developing smut-resistant (Table 3). High individual and family heritabilities of 0.56 and 0.84, respectively, for smut resistance was probably the major factor responsible for the rapid progress observed in Hawaii (Wu *et al*[41]). Because of these high heritabilities, it is less important to precisely define a resistant smut reaction in terms of percent infection.

Smut races

The evidence reported indicates the presence of smut races A and B in Hawaii (Comstock and Heinz[5]), at least two races in Brazil (da Silva and Sanguino[8], Toffano[38]), two races in Taiwan (Hsieh and Lee[13], Leu and Teng[21]), and five races in Pakistan (Muhammad and Kausar[26]). Other countries did not report races. It is not clear to what extent races commonly occur in different countries.

Since 1971, the Hawaiian Sugar Planters' Association has been actively involved in a cooperative smut disease testing program with Brazil, Jamaica, Rhodesia, Taiwan and the Philippines. Varieties were shipped to these countries and their smut reactions determined. Table 4 summarize a partial list comparing smut reactions of near-commercial Hawaiian varieties in several countries to races A & B in Hawaii, the data show that the organisms occurring in various countries are not closely related to races A or B, suggesting that different races of the smut pathogen exist in each of the different countries.

Differing varietal reactions observed in several countries and reported in the literature also suggest the existence of races. For example, variety Co 421 was reported susceptible in India and resistant in Natal, Brazil, and Kenya (Robinson[29]). Variable smut ratings were reported for variety B 52107 in Brazil, Hawaii, Kenya, Guyana, and the Philippines (Whittle[40]). In Rhodesia, variety Co 462 was originally immune, while on CP 29-116 smut was only rarely observed. These varieties are now rated as susceptible to smut (James[17]). In Taiwan, the evidence has been that races 1 and 2 can be hybridized to produce a third race with a different host range (Hsieh and Lee[13], Leu[23]).

Part of the variation may be explained on the basis of variety-environment interaction as observed in Hawaii in our 8 years of smut testing in various localities and environments at different times of the year. The same kind of experience has been reported from India and Rhodesia (Bachchhar and Patil[3], Durairaj *et al*[9], Jaleel Ahmed and Sambasivam[16], James[17], Sharma

TABLE 4. Comparison of smut reactions of 11 Hawaiian varieties in several countries

Country	Varietal Smut Reaction[a]										
	44–3098	50–7209	53–1447	54–775	57–5174	59–2122	60–1102	60–3865	60–4675	60–5657	64–4499
Brazil	S	I	S		I	S		I	S		S
Jamaica	S	S		R	S					S	
Philippines	S	S	S	R	S		S	S	R	S	S
Rhodesia	S	S	R	R	R	R	R	R		I	
Taiwan	S	R	S	S		R	R	R	R	R	I
Hawaii											
race A	S	R	S	I	R	R	S	R	R	R	R
race B	S	S	S	I	S	S	R	R	R	I	S

[a] *R = resistant or ~15% infection*
I = intermediate or 16-25% infection
S = susceptible or 26-100% infection

TABLE 5. Comparison of expected and observed resistant and susceptible reactions to races A and B of 753 near-commercial Hawaiian varieties

Reaction to race			Number[a]	
A	B		Expected	Observed
R	R		502	507
R	S		230	226
S	R		14	9
S	S		7	11
		Total	753	753

[a] $\chi^2 = 3.29,\ .05 < p < .10$

et al.[32]). Still, more extensive and standardized testing in different localities is required before concluding that smut races differ from country to country.

Another issue with respect to smut races has emerged from observations

TABLE 6. Comparison of expected and observed resistant and susceptible reactions to races A and B of 280 seedling progeny from 40 biparental crosses

Reaction to race		Number[a]	
A	B	Expected	Observed
R	R	88	87
R	S	129	125
S	R	26	26
S	S	37	42
	Total	280	280

[a] $\chi^2 = .655, .25 < p < .50$

of varietal reactions to races A and B in Hawaii. Gene frequencies for resistance and susceptibility to races A and B were calculated from observed varietal reactions and used to calculate expected frequencies assuming independent gene action. Data (Comstock and Ferreira[6]) were obtained from populations consisting of 753 near-commercial varieties (Table 5) as well as 280 seedling progeny from 40 biparental crosses (Table 6). Resistance to race A is unrelated to that of race B, suggesting smut resistance to individual races is inherited independently. Further analysis utilizing a larger number of progeny from fewer specific crosses may conclusively elucidate the gene action involved.

DISCUSSION

As previously seen, techniques of testing and evaluating varietal smut reactions are highly variable from country to country. To recommend that a standard procedure be followed by all workers is premature at this time. Much is not understood about the mechanism of resistance of sugarcane to smut; there is no understanding on which to base decisions concerning the characters contributing to resistance. Until more is understood about the mechanism of resistance, standardizing methods of testing and evaluating resistance is not recommended. In addition, different countries have varying needs in terms of levels of resistance required. A country with an abundance of desirable resistant lines, can be more stringent, with respect to smut resistance, in its variety evaluation program. What would be considered undesirable for one country might be acceptable in another country when it cannot afford the luxury of discarding much of its progeny from various crosses.

Although each country may vary its smut testing and evaluation techniques, certain inoculation procedures should be defined. For example, variety from which inoculum was derived, inoculum concentration, method of inoculation, preplant treatment, and age of crop at time of disease reaction evaluation should be clearly stated. In evaluating varietal reactions, the different ratings

and percent infection categories must be clear. In this way, an individual can determine varietal reactions to suit the particular needs in his area and not be confused by numerical grades which differ in their meaning from country to country.

Since the data presented concerning the existence of races are only suggestive, there is a need to standardize smut testing so that reliable data will be available. Standardization should occur with the use of standard varieties and uniform inoculum. At this time, suggestions can only be made regarding certain varieties that differ widely in smut reaction. Variety H44-3098 appears to be susceptible whereas it has been tested. In Hawaii, H50-7209 is resistant to race A and susceptible to race B. A similar observation with variety F 134 and races 1 and 2 has been made in Taiwan. Variety Co 421 appears highly variable in its smut reaction depending upon the country in which it is tested. The same is true for B-52107. Variety POJ 2878 has been reported highly susceptible in the Philippines, but practically immune in Brazil and Argentina (Cross[7], Rivera[28]). In Hawaii, H68-1158 is highly resistant to races A and B. Smut has never been observed on CP 63-588 (Norman James, personal communication). Many, if not all, of these varieties may prove valuable as differential varieties. As data are accumulated, appropriate changes in the set of differentials may be made.

Because races of smut probably exist and resistance to different races is probably unrelated, the successful use of resistance depends upon our ability to recognize different smut races. Only then can we be certain that the different races from a geographical area are used in screening seedlings to develop resistant varieties. It would be best to maintain inoculum sources of the various races on appropriate differential host varieties. The whip farms can be ratooned or plowed as needed, but inoculum provided would be reasonably constant.

It may prove useful also, especially in regions where smut has been introduced in the last 10-15 years, to initiate a monitoring program to identify new smut races as they develop. Nurseries of highly resistant material could be planted in areas of highest disease activity. When smut is discovered on resistant clones, it would suggest that a new race exists. Appropriate testing of inoculum produced on the previously resistant clone would indicate if there were a new race of smut.

There have been many suggestions made. Ideally, if they were all followed, much progress could be made in learning about smut races and their dynamics. On the other hand, it may not be reasonable to expect total standardization of techniques and interpretation of data. What we hope for, at least, is that more consideration and effort be made at understanding the race picture of culmicolous smut of sugarcane.

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EVALUACION DE CAÑA RESISTENTE AL "TIZON"

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RESUMEN

Desde que el mecanismo de resistencia es el más importante en la estrategia de control del "tizon" de la caña de azúcar (*Ustilago scitaminea* Sydow), se han revisado en diferentes paises procedimientos de inoculación y evaluacion de variedades resistentes. Además se ha presentado data sugiriendo que existen en general diferentes razas del "tizon" y que por ejemplo en Hawaii la herencia de la resistencia a la raza A es independiente de la raza B. Se han hecho recomendaciones para standarizar ciertos procedimientos para probar y reportar reacciones del "tizon" a la variedad. Esto podria ayudar a contestar hasta cierto punto preguntas de cuales razas existen, y puede proveer datos sobre la dinamica de estas razas.

REACTION OF MISCANTHUS GERMPLASM TO DOWNY MILDEW AND CULMICOLUS SMUT OF SUGARCANE

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ABSTRACT

The genus *Miscanthus* indigenous to Taiwan is highly resistant to downy mildew *(Sclerospora sacchari)* and culmicolus smut *(Ustilago scitaminea)* diseases. As to the reaction of 129 clones to downy mildew 18 (13.9%) had low percentage of infection while the remaining 111 clones (86.1%) were not infected. Similar results were obtained with both *M. sinensis* and *M. floridulus.* At the same time resistance to culmicolus smut was continuously determined for both plants and ratoons of 124 clones in 3 years. None of them was found susceptible.

INTRODUCTION

In response to the proposal made by the ISSCT Committee on Germplasm and Breeding for recommendations on collecting sugarcane germplasm (Daniels *et al*[1]), an island–wide *Miscanthus* germplasm collection has been conducted by the Taiwan Sugar Research Institute (TSRI) since 1975 (Lo *et al*[5]).

During the past three and half years, intense researches were carried out on the morphology, flowering, chromosome counting, isozyme patterns and downy mildew and smut disease resistance of the collected germplasm. In 1978, 26 clones, of *M. sinensis* and *M. floridulus* were used as male parents and crossed with commercial cultivars. Approximately 7,800 seedlings were single planted to the fields.

Downy mildew *(Sclerospora sacchari* Miyake) and culmicolus smut *(Ustilago scitaminea* Sydow) are major disease of sugarcane in Taiwan. *Miscanthus,* however, has been considered to be resistant to both cane diseases (Daniels *et al*[1]). Testing the resistance of the *Miscanthus* germplasm collection to these diseases is a part of *Miscanthus* project at Taiwan Sugar Research Institute.

MATERIALS AND METHODS

1. *Test of reaction to downy mildew*

In the past 3 years, the reaction to downy mildew of 129 clones of *Miscathus,* consisting of 83 clones of *M. sinensis,* 42 of *M. floridulus,* 1 of *M. flavidus,* and 3 unidentified, were determined at Nan-Tou Trial Field. Diseased sugarcane

plants were planted in every 4 rows of the *Miscanthus* (Leu, Wang Hsieh and Tzean[3]). Number of diseased stalks was investigated monthly from June through November. Percentage of disease infection was obtained by dividing the total number of diseased stalk with total number of stalk per clone.

2. *Test of reaction to smut*

Reaction to culmicolus smut of 124 clones of *Miscanthus*, consisting of 81 *M. Sinensis*, 39 *M. floridulus*, 1 *M. flavidus*, and 3 unidentified, were tested at Shi-Chung-Chi Trial Field. Because no buds on the stalks of *Miscanthus* plants were available for wound-paste inoculation, the leaf sheaths of 10 young shoots of each stool of *Miscanthus* and the control plants were prickled and brushed with thick teliospore suspension of race 1. They were then covered overnight with polyethylene bags. Smut disease was investigated periodically.

RESULTS AND CONCLUSION

1. *Downy mildew*

The *Miscanthus* germplasm native of Taiwan has been satisfactorily resistant to downy mildew. Only 13.9% (18 clones) of the tested clones were susceptible to the disease, the rest 86.1% (111 clones) were resistant (Table 1). Tests conducted in 1977 to 1978, on the other hand, showed that 30.8% of the total 390 clones under study were moderately to highly resistant (Lee, Wag and Wsieh[2]). This means that *Miscanthus* germplasm in Taiwan has developed high resistance to downy mildew. Nearly 15% of each species, 12 out of 83 of *M. sinensis* and 6 out of 42 *M. floridulus*, were infected by the disease.

TABLE 1. Reaction of *Miscanthus* to downy mildew

Infection	Number of clones				Total	%
	M. sinensis	*M. floridulus*	*M. flavidus*	unidentifield		
0	71	36	1	3	111	86.1
0.1–10	10	5	0	0	15	11.6
10.1–30	2	1	0	0	3	2.3
30.1–100	0	0	0	0	0	0
Total	83	42	1	3	129	100

TABLE 2. Reaction of ***Miscanthus*** sugarcane variety and other plants to culmiculus smut

Infection	Number of clones						
	M. sinensis	*M. floridulus*	*M. flavidus*	unidentified	NCo310	*S. spontaneum*	*Erianthus*
0	81	39	1	3	0	7	4
0.1–10	0	0	0	0	0	1	0
10.1–30	0	0	0	0	0	2	0
30.1–45	0	0	0	0	1	0	0
45.1–100	0	0	0	0	0	0	0
Total	81	39	1	3	1	10	4

2. *Culmicolus smut*

There were four plantings and two ratoons of *Miscanthus* in 3 consecutive years. Each crop was inoculated with strain 1 smut (Leu and Teng[4]). It has been shown that Taiwan *Miscanthus,* is highly resistant to smut disease (Table 2). NCo310, *Saccharum spontaneum Erianthus,* were used as controls. Each of them was more or less susceptible. This indicated that the use of the Taiwan *Miscanthus* as a germplasm for smut resistance would be highly evaluated.

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REACCIONES DEL *MISCANTHUS* GERMOPLASMA AL AÑUBLO LANOSO Y AL CARBON CULMICOLUS DE LA CAÑA DE AZUCAR

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RESUMEN

Se reveló que el genero *Miscanthus* indigeno en Taiwan es altamente resistente a las enfermedades del añublo lanoso y del carbon culmicolus. Entre 129 clones evaluados, 18 (13.9%) mostraron bajo porcentaje de infeccion, los demas 111 clones (86.1%) no estaban infectados Tanto *M. sinensis* como *F. fluridulus* demonstraron los mismos resultados en este aspecto. Al mismo tiempo se efectuaron estudios de resistencia al carbon culmicolus por un periodo de tres años y para ambos plantilla y retoño en 124 clones y en ninguno se encontró susceptibilidad.

SUGARCANE SMUT (*USTILAGO SCITAMINEA* SYD) IN BELIZE AND MEXICO

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ABSTRACT

Fifty sugarcane varieties were screened for resistance against smut fungus, *Ustilago scitaminea* Syd. Inoculation was done by dipping the seedpieces in spore suspension (5×1^6/ml) for 30 minutes. Of these clones, 10 were highly susceptible, 5 susceptible, 4 moderately resistant and 31 resistant. Whips emerged within 60 to 90 days after planting. Varieties POJ 36, Co 213, Mex 57-337, and NCo 310, grown commercially in Mexico, showed grassy appearance and were highly susceptible to the smut.

INTRODUCTION

Sugar cane smut caused by *Ustilago scitaminea* was discovered 29 July 1978 in San Antonio, Belize, 30 kilometers from the Mexican border. The disease has been found in small scattered patches around the cane belt on BJ 57-27 varieties, since its discovery on HJ 57-41 variety (Flores[3]). It may become a menace to all Central America because it keeps on spreading during the last 3 years to Jamaica (1976), Florida, USA, Belize and Cuba (1978) and recently in Honduras and Nicaragua (Dean and Miller[2]).

The area planted to sugarcane in Belize is about 20,000 hectares and produces over 1,000,000 long tons of cane. The principal commercial varieties grown are POJ 2878 (40%), HJ 57-41 (20%), BJ 57-21 (14%), BJ 59-27 (9%), and B 52-298 (9%). Control measures initiated by the Belize Sugar Industry Research Station officers include inspection of the fields of diseased stools, roguing and burning of infected plants, replacing susceptible varieties with resistant ones, and screening of varieties under natural conditions (Fors[4]). The Mexican Sugar Institute (IMPA) taking advantage of this situation in September, 1978 has inoculated and planted in this center 50 of their commercial and promising canes in order to know the reaction of the Mexican varieties and the races of smut fungus prevalent in Jamaica, Belize and Florida. Commercial varieties currently grown in more than 100,000 hectares in Mexico are POJ 36, Co 213, Co 290, Co 421 and NCo 310 which are highly susceptible to the smut.

The experiment was set up under field conditions in Tower Hill, Belize

in September, 1978 to determine the relative resistance or susceptibility of 50 sugar cane varieties to the smut.

MATERIALS AND METHODS

Single-eyed cuttings of each variety were prepared and dipped for 30 minutes in spore suspension of *U. scitaminea,* with a density of 5 $x10^6$/ml water. All inoculated seedpieces were incubated for 24 hours at approximately 28°C before planting in the field (Ladd and Heinz[5]).

Twenty seedpieces from each variety were planted per row, with rows 1 m apart. Normal agronomic practices were carried out. Observation on the incidence of smut were recorded weekly until 180 days after planting (March 1979). The evaluation of reaction of the varieties was based on percentage of infected stools with whips out of the total number of stools in each row following the scale used by Burgess[1] in Jamaica:

Stools infected Percentage	Reaction
0 – 5	Resistant (R)
5.1 – 15	Moderately resistant (MR)
15.1 – 30	Susceptible (S)
More than 30	Highly susceptible (HS)

Highly resistant clones POJ 2878 and B 4362 and highly susceptible clones POJ 36 and Co 213 were included in the test as checks. All the varieties from this test were cut 180 days after planting for observation of reaction in ratoons.

RESULTS AND DISCUSSION

Table 1 lists the 50 varieties used and their reaction to smut. Ten clones were highly susceptible: 5 susceptible, 4 moderately resistant, and 31 resistant. It was observed that in the highly susceptible and susceptible clones, emergence of whips was earlier, i.e. within 60 days after planting on B 49-119, Co 213, Co 290, Mex 56-541 and POJ 36; in the moderately resistant and resistant clones, whip emergence was delayed. The varieties POJ 36, Co 213, Mex 57-337 and NCo 310 showed grassy appearance due to the diseased shoots; these symptoms were typical in clones which are highly susceptible.

The period for the emergence of whips was from 60 to 90 days after planting, and it is similar with the findings of workers in several countries. It will be important for the Mexican and Belize sugar industry to determine how many races of smut are prevalent in these countries. Screening tests helps to eliminate susceptible varieties and to replace them with resistant ones. Screening for smut resistance has been carried out for the first time in Belize and Mexico because the disease was found last year. These tests will be continued for all the commercial varieties and in our breeding program. It is also convenient

TABLE 1. Reaction of 50 sugarcane varieties to smut infection. (Tower Hill, Belize)

Variety	% Infection	Reaction	Variety	% Infection	Reaction
B 3439	27.0	HS	Mex 57-337	60.0	HS
B 4362	9.0	MR	Mex 57-473	0.0	R
B 43-337	0.0	R	Mex 57-683	0.0	R
B 49-119	33.0	HS	Mex 57-1285	4.0	R
Co 213	73.0	HS	Mex 58-418	0.0	R
Co 290	30.0	HS	Mex 59-32	0.0	R
Co 331	16.0	S	Mex 60-207	55.5	HS
Co 419	8.0	MR	Mex 60-445	38.8	HS
Co 421	66.6	HS	Mex 60-1403	0.0	R
CP 29-203	30.0	S	ITAV Mex 60-1329	20.0	S
CP 44-101	23.0	S	Mex 60-1429	0.0	R
H 37-1933	9.0	MR	Mex 64-213	25.0	S
H 44-3098	22.0	S	Mex 64-1214	0.0	R
L 60-14	64.0	HS	ITAV Mex 66-339	0.0	R
Mex 52-17	0.0	R	Mex 66-446	36.8	HS
Mex 54-81	72.7	HS	Mex 69-401	30.0	HS
Mex 55-261	42.0	HS	Mex 69-418	19.4	S
Z Mex 55-32	13.0	MR	Mex 69-420	13.3	MR
Mex 55-250	55.0	HS	Mex 70-421	0.0	R
Mex 56-18	0.0	R	NCo 310	52.0	HS
Mex 56-363	20.8	S	PM 35	0.0	R
Mex 56-476	100.0	HS	POJ 36	62.0	HS
Mex 56-441	12.0	MR	POJ 2878	0.0	R
Mex 57-29	14.0	MR	Q 77	0.0	R
ITAV Mex 57-197	69.5	HS	US 52-171	54.0	HS

to determine the races of the fungus prevalent in Belize, Jamaica and Florida, USA.

CONCLUSIONS

Fifty commercial and promising cane varieties from Mexico were screened for resistance against smut. Of these varieties 10 clones were highly susceptible, 5 susceptible, 4 moderately resistant and 31 resistant.

Emergence of whips was observed earlier in highly susceptible varieties within 60 days after planting. Varieties POJ 36, Co 213, Mex 57-337 and NCo 310 showed grassy appearance as a result of infection.

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EL CARBON DE LA CAÑA DE AZUCAR EN BELIZE Y MEXICO

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RESUMEN

El carbon de la caña (*Ustilago scitaminea* Sydow) se descubrió en julio de 1978, en Belize cerca de la frontera mejicana en la variedad HJ 57-41. Esto representa una amenaza para toda Centro America y la industria azucarera de Mexico ya que la epidemia de carbon se ha estado diseminando durante los ultimos 3 años de Jamaica, Florida, USA, Cuba, Honduras y Nicaragua. El Instituto de Azucar Mexicano establecio un experimento en Tower Hill, Belize para separar 50 variedades resistentes al carbon. Los clones fueron inoculados con una concentracion de 5 x 10^6/ml de agua. De los 50 clones separados 10 eran altamente susceptibles, 5 susceptibles, 4 moderadamente resistentes y 31 resistentes. El periódo de emergencia de los ("whips") era de 60-90 dias despues de siembra. POJ 36, Co 213, Mex 57-337 y NCo 310, variedades comerciales en Mexico mostraron una apariencia ("grassy") y fueron altamente susceptibles al carbon.

SCLEROSPORA SPECIES CAUSING DOWNY MILDEW OF SUGARCANE *(SACCHARUM OFFICINARUM* L.) IN THE PHILIPPINES

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ABSTRACT

Three species of *Sclerospora* were identified from 12 isolates obtained from mildewed sugarcane and *Saccharum spontaneum* collected from selected sugarcane-growing provinces – Negros Occidental, Pampanga, Tarlac and Laguna. Based on symptomatology, gross morphology and virulence, three *Sclerospora* species were identified, namely: *Sclerospora philippinensis* Weston, *Schlerospora sacchari* Miyake and *Sclerospora spontanea* Weston.

The characteristic symptoms produced by each of the three *Schlerospora* species on standard test cane (Phil 58260) showed distinctive patterns. *S. philippinensis* showed a whitish discoloration only on the base of the young leaves and up to the tip of succeeding leaves without leaf shredding, while *S. sacchari* produced well-defined continuous chlorotic streaks on the leaf blade with characteristic leaf shredding and stunting of the affected plant. The symptoms manifested by *S. spontanea* were the same as those of *S. sacchari,* except for the presence of light yellow, irregular and discontinuous streaks and absence of growth stunting.

Morphologically, highly significant differences in length and width of conidiophores and in conidial length among the three *Sclerospora* species were observed. Conidia of *S. philippinensis* were elongate-oval or ellipsoid, while those of *S. sacchari* were elliptical or oblong with rounded apex and slightly apiculate base. *S. spontanea* slightly differed from *S. sacchari* in conidial shape. Conidia of *S. spontanea* were more elongately ellipsoid or cylindrical with round to acute *apices.*

Of the three *Sclerospora* species, *S. philippinensis* was the most

virulent on six *sugarcane* varieties, with a mean systemic infection of 28.36% on all test canes, while *S. sacchari* and *S. spontanea* had 14.04% and 12. 07%, respectively. The mean systemic infection differences between the two latter species was not statistically significant.

INTRODUCTION

Of the downy mildew organisms attacking some members of Graminae family in the Philippines, genus *Sclerospora* is one of the most confusing group among peronosporaceous fungi. Various investigators opine that *Sclerospora* of sugarcane reported from the Philippines is a far less virulent strain or possibly a different species. Dr. K. C. Ling (Personal Communication, 1969) commented that *Sclerospora* species causing downy mildew of sugarcane in he Philippines is induced by *S. miscanthi.*

The first attempt to investigate the difference between *S. philippinesis* and *S. sacchari* was done by Elazegui and Exconde[5]. Morphological characteristics were the parameters used in differentiating these two *Sclerosporas.* These workers found marked differences in shape and length of conidia. This finding led them to conclude that these two species are distinct from each other.

A recent investigation of the development, morphology, cytology and symptomatology of Philippine *Schlerosporas* was done by Visarathanonth[12]. His experiment involved conidial inoculation of susceptible corn plants with *S. philippinensis, S. spontanea, S. sacchari* and *S. miscanthi.* Results showed that only *S. philippinensis* and *S. sacchari* infected corn. Corn plant infected with *S. sacchari* showed discontinuous, irregular chlorotic streaks not arising from the base of the leaves. Chlorotic streaks produced by *S. philippinensis* always appeared from the base of the leaves. Infected leaves of *Miscanthus* and *Saccharum spontaneum* also exhibited chlorotic streaks from the base and became reddish brown with age. The size of conidiophores and conidia of *S. philippinensis* and *S. sacchari* on corn plant were not distinct. The conidiophores of *S. miscanthi* and *S. spontanea* were shorter than those of *S. philippinensis* and *S. sacchari,* The hyphae, conidiophores and conidia of the four species were all multinucleate.

Leece[9] reported that *S. spontanea* and *S. philippinensis* resembled *S. Sacchari* very closely in morphoplogical features.

Rivera[10] observed the serious outbreak of downy mildew of sugarcane among local hybrid seedlings from 1956 to 1958 at the breeding lots of La Granja and Cadiz Stations in Negros Occidental. He noted that infection of sugarcane seedlings reached as high as 75% particularly on hybrids of *Saccharum spontaneum* L. parentage. After these years there was no report of any serious occurrence of the disease except a severe natural infection of local hybrid seedlings, including some varieties, at La Granja Sugarcane Experiment Station in 1970. Up to the present time this disease is a problem most especially on first-year seedlings.

The losses due to this disease are directly related to the resistance of the varieties being grown, and the mere presence of the disease precludes the commer-

cial exploitation of susceptible but otherwise desirable varieties. This direct loss, although difficult to assess, can often reach considerable proportions.

Growing resistant varieties is the most effective and economical measures to control downy mildew. The cane breeder and the plant pathologist are working hand in hand to produce such varieties and to integrate other agronomic practices in order to restrain the disease. However, technical difficulties had been encountered in the screening of varieties for resistance to downy mildew because of lack of information on species of *Sclerospora* attacking sugarcane under field conditions and on the diagnostic symptoms induced by each species on the affected plants. This study was therefore conducted with a view of contributing to knowledge on downy mildew of sugarcane.

This work was conducted to identify the *Sclerospora* species attacking sugarcane in the Philippines, ascertain the symptoms produced by these species on sugarcane and study the gross morphology of different downy mildew from sugarcane.

MATERIALS AND METHODS

Collection and maintenance of isolates

Cuttings of sugarcane with downy mildew were collected in localities from selected sugarcane-growing provinces of the Philippines. In addition, cuttings of downy mildew-infected *Saccharum spontaneum* were collected from La Granja, La Carlota City and Sagay, Negros Occidental. These cuttings were planted in sterilized compost soil in earthen pots and kept in isolation cages to prevent possible infection from air-borne conidia of *Sclerospora*.

After examination of the conidia and comparison of the symptom pattern of the original mildewed plants, three groups were clearly recognizable. Hence, one isolate from each group was retained for detailed study. These were isolate 1 on sugarcane from La Granja, isolate 5 on sugarcane from Sagay and isolate 11 on *Saccharum spontaneum* from Sagay.

Host plants

Yellow flint corn (UPCA Var. 3) and cane variety Phil 58260 were used as host plants for single spore and mass spore inoculations to different *Sclerospora* isolates, respectively.

Pregerminated corn seeds were planted in sterilized compost soil placed in plastic cups. After planting, all the seedlings were placed in a growth chamber with a temperature-relative humidity combination of 30°C/75% RM for "day" condition and 23°/975 RM for "night" condition (Barredo[1]).

Healthy sugarcane cuttings were planted in sterilized compost soil. At two-to

three-leaf stage all the seedlings were inoculated and subjected to the same condition as described above.

Inoculum

Leaves of downy mildew-infected plants were gathered from the inoculum source between 5 and 6 p.m. Each leaf was washed in running water and swabbed with sponge to remove the original downy growth. At 8 p.m. the washed materials were placed in clean petri plates, with the lower surface facing the bottom of the plate, covered with moistened plastic bags and incubated in the growth chamber at 23° C and 97% rh. The newly formed conidia were harvested after 1 to 8 hrs. of incubation. Inoculum suspension was adjusted to 50,000 ± 1,000 conidia per ml with the use of hemacytometer (Barredo[1]).

Single spore inoculation

Sugarcane leaves infected with *Sclerospora* from La Granja (Isolate 1) were prepared for sporulation. Monospore culture of the isolate was done by pouring a thin layer of sterilized two-percent-water agar into a petri dish to which diluted conidial suspension of the isolate was then poured. These plates were examined under a stereoscopic dissecting microscope, and the slightly germinated conida were marked. With the use of sharp finely pointed coconut-midrib, a small agar block containing a conidium was carefully picked and then inserted into the whorl of a five-day old susceptible corn plant. A small drop of distilled water was previously placed in the whorl to dislodge the spore from the agar block and also to provide moisture for subsequent development of the conidium (Dogma *et al*[3]). The inoculated plants were kept inside the growth chamber under "night" condition of 23°C/97% rh for 8 hrs before transferring them to the isolation cages for observation.

The systemically infected corn plants were maintained inside the isolation cages and served as the homogenous isolate obtained from the original collection.

Mass spore inoculation

The chlorotic leaves of corn infected with single conidium of isolate 1, chlorotic leaves of sugarcane (isolate 5) and *Saccharum spontaneum* (isolate 11) from the original collection were prepared for sporulation. The conidial suspension was injected into the spindle of sugarcane seedlings with the use of hypodermic needle. The inoculated plants were kept inside the growth chamber under "night" condition of 23 °C/97% RH for 24 hr and 4 days thereafter with alternate "day" and "night" conditions. After this period all plants were transferred to the isolation cages for further incubation.

Symptomatology

Standard cane variety Phil 58260 artificially infected with the different *Sclerospora* isolates was used in this study. During the first six weeks the plants

were placed in the isolation cages and then transferred under field condition for observation. The following were the criteria used in symptom description: (1) type of symptoms; (2) presence or absence of discoloration; (3) presence of leaf splittings; and (4) characteristic growth.

Gross morphology of the isolates

Leaves of standard test cane systemically infected by three distinct isolates of *Sclerospora* were thoroughly washed and swabbed with a rubber sponge to remove the original downy growth. These were cut into sections, each 6 to 7 cm long and taped separately under the petri plate cover with the lower side of the leaves facing the plate bottom.

The petri dishes were placed in moist plastic bags and kept inside the growth chamber adjusted to condition favorable for sporulation (23^{O} C/97% RH, "night" condition). After 8 hr, dislodged spores were collected and fixed in Farmer's solution and kept in small specimen vials. From these 100 conidial measurements (length and width) of each of the isolate were made. At the same time, leaf sections each 1 cm long, were cut and fixed in Farmer's solution, cleared in plain lactophenol and stained in lactophenol cotton blue whenever conidiophore measurement had to be made. From these excised leaves 100 conidiophores were measured.

Test of virulence of isolates and reaction of differential cane varieties

Twenty healthy single-eye cuttings each of Badila, Phil 58260, Phil 6317, Phil 6425, Phil 6429 and Phil 6607 sugarcane varieties were planted separately in plastic bags, or a total of 360 plastic bags.

Inoculation was done 14 days after planting. After these periods the inoculated plants were transferred to, and kept in, isolation cages for six weeks and then finally transferred under field condition until they were six months old.

Reactions of each variety were based on the percent systemic infection (Titatarn & Exconde[11]). with slight modification, following the International Rating Scale as suggested by Hutchinson[8]. These are: 0-15% infection (2 or resistant); 16-45% infection (5 or intermediate); and 46-100% infection (8 or susceptible).

RESULTS

Symptomatology

Each of the three *Sclerospora* species produced distinct symptom pattern on sugarcane.

S. philippinensis. The first symptoms appeared on the young leaves as water-soaked, more or less circular lesions 3 to 5 days after inoculation. Ten days later a pale yellow to whitish discoloration appeared on the base of the 4th and 5th leaves and extending up to the middle portion of the lamina (Fig. 1). As the disease

advanced, a large portion of the succeeding leaves exhibited a pale yellow to whitish discoloration. Emerging shoots showing systemic symptoms were quickly followed by the production of white downy growth, which was the most diagnostic feature of the disease. Sporulation which occurred on the nether leaf surface was found not only on the discolored area but also on the normal green portions in between the discolored parts. Diseased plant produced thinner stalk but not stunted and no

FIGURE 1. Leaf portions of standard test cane (Phil 58260) infected with *S. philippinensis* showing whitish discoloration (A-diseased; B-healthy)

FIGURE 2. Leaf portions of standard test cane (Phil 58260) infected with *S. sacchari* showing well-defined clorotic streaks (A-diseased; B-healthy)

reddish discoloration nor shredding of the leaves exhibited by the mildewed canes even during the later growth stage of the plant. Microscopic examination of the diseased leaves revealed the absence of oospores.

S. sacchari. Initial symptoms appeared as pale yellow to whitish discoloration on the young leaves 3 to 5 days after inoculation. One week after inoculation numerous reddish specks were observed on the discolored area. As the plant grew, the few succeeding leaves appeared apparently healthy. However, greenish yellow streaks running parallel to the veins were seen on the leaf blade of the main stalk 9 weeks later. The streaks were generally distributed across the whole width of the leaf, however, in a few cases they were confined to half of the lamina lengthwise. The streaks were separated by normal green tissue of variable width. The length of the streaks ranged from a few centimeters to several inches and often extend practically the full length of the leaf. The width of individual streaks varied from 1 to 3 mm (Fig. 2). Short white to yellowish streaks were also observed on the leaf blades of emerging secondary tillers.

The streak formation was quickly followed by the production of white downy growth, especially during warm and humid nights. Sporulation was confined only to the nether surface of the chlorotic area, however, in some cases it also occurred on the upper surface opposite the lesion.

With age, the color of the streak changed from greenish yellow to yellow, then to reddish brown and finally to dark red. Later, the tissues which became red rapidly shredded. Shredding started from the tip and extended to the base of the leaf. Microscopic examination of the shredded leaves revealed the presence of numerous brownish oospores which were the sexual spores of the causal agent.

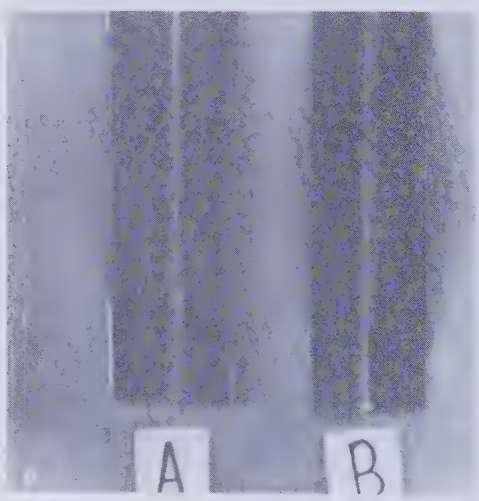

FIGURE 3. Leaf portions of standard test cane (Phil 58260) infected with *S. spontanea* showing light yellow, and discontinuous streaks (A-diseased; B-healthy)

Some diseased plants were stunted, with narrow and rolled leaves, abnormally thin stalk and less number of tillers.

S. spontanea. The early symptoms of the disease appeared as a few pale green stripes parallel to the leaf veins. Later on, these stripes progressed and became light yellow, irregular and discontinuous (Fig. 3). Most of the stripes were confined near the midrib. The width of individual stripes varied from 1 to 5 mm. The color of the stripes remained unchanged with increasing plant age. However, as the disease advanced, the succeeding leaves exhibited a symptom pattern exactly similar to that manifested by the plants inoculated with *S. sacchari.*

During warm and humid nights downy growth consisting of conidiophores and conidia of the causal fungus were produced on the lower side of the diseased leaves.

Leaf shredding was also observed when the mildewed canes were about five months old. Microscopic examination of the shredded leaves showed numerous brownish oospores. However, there was no growth stunting.

Gross morphology of the species

The gross morphology of conidia and conidiophores of each of the three different *Sclerospora* species from systemically infected Phil 58260 cane variety was studied.

S. philippinensis. Conidia were hyaline, spherical when young and elongate ovoid when mature. Measurements of 100 conidia ranged form 20.91 to 42.94μ in length and 10.25 to 19.68μ in width or an average of 29.34 x 14.64μ (Table 1). In length, 85% of the conidia measured fell within the limit of 24 to 34μ and in width, 83% fell within the limit of 12 to 18μ (Table 2).

The conidiophores were hyaline, erect, thin walled, finely granular, arising singly or in group from the stomata and dichotomously branched usually with two primary branches on which 2 to 5 secondary branches were formed. The length ranged from 26.2 to 396.4μ and the width from 10 to 14.8μ with an average of 340.2 x 12.9μ (Table 1).

S. sacchari. The conidia were myaline elliptical or oblong with round apex and slightly apiculate base, thin-walled and smooth. Conidial length ranged from 31.16 to 52.89μ and the width from 9.43 to 19.27μ with no range of 42.47 x 14.06μ (Table 1). In length, 68% of the conidia measures fell with the range of 40 to 50μ while in width, 81% fell within the range of 10 to 16μ (Table 2).

The condiophores were myaline, erect, arising singly or in groups from the stomata and dichotomatously branched, two primary branches on which five to four secondary, branches were formed. The length ranged from 190.8 to 432.4μ and the width from 7.2 to 14.8μ cr an average of 268.65 x 10.57μ (Table 1).

S. spontanea conidia were hyaline and elongately ellipsoid with round to

TABLE 1. Average size of 100 conidia and conidiophores of different *Sclerospora* species taken from systemically infected Phil 58260

Species	Conidia		Conidiophores	
	Length	Width	Length	Width
S. philippinensis	29.34	14.64	340.20	12.90
S. sacchari	4.247	14.06	268.65	10.57
S. spontanea	46.72	12.28	325.25	15.22
LSD: .05	—	1.40	—	—
.01	1.52	—	38.22	1.37

TABLE 2. Distribution of measurements (length and width) of 100 conidia each of three *Sclerospora* species from artificially infected Phil 58260

Class limit	Species and Length			Class limit	Species and Width		
μ	*S. philip-pinensis*	*S. sac-chari*	*S. spon-tanea*	μ	*S. philip-pinensis*	*S. sac-chari*	*S. spon-tanea*
20-22	1	0	0	6-8	0	1	1
22-24	4	0	0		0	1	21
24-26	14	0	0	8-10	7	15	28
26-28	13	0	0	10-12	34	39	37
28-30	33	0	0				
30-32	18	1	1	12-14			
32-34	7	7	0	14-16	36	27	8
34-36	6	6	2				
36-38	1	5	2	16-18	13	11	4
38-40	1	7	5	18-20	10	7	1
40-42	1	27	7				
42-44	1	12	16	20-22	0	0	0
44-46	0	15	17		0	0	0
46-48	0	6	17	22-24	0	0	0
48-50	0	8	9	24-26	0	0	0
50-52	0	4	10		0	0	0
52-54	0	2	6	26-28	0	0	0
54-56	0	0	4				
56-58	0	0	1	28-30			
58-60	0	0	1				
60-62	0	0	2	30-32			

TABLE 3. Percent systemic infection and reactions of six different cane varieties to three *Sclerospora* species under field conditions

Species	Sugarcane varieties												Mean
	Badila		Phil 58260		Phil 6317		Phil 6425		Phil 6429		Phil 6607		
	% Inf./$R^{o}X^{a}$		% Inf./$R^{o}X^{a}$		% Inf./$R^{o}X^{a}$		% Inf./$R^{o}X^{a}$		% Inf./$R^{o}X^{a}$		% Inf./$R^{o}X^{a}$		
S. philip-pinensis	0	2	58.5	8	26.46	5	18.44	5	53.73	8	12.92	2	28.36
S. sacchari	0	2	39.23	5	0	2	0	2	45.00	5	0	2	14.04
S. spontanea	0	2	33.21	5	0	2	0	2	39.23	5	0	2	12.07
Mean	0		43.65		8.85		6.15		45.99		4.31		

LSD: .01 = 10.28 (species)

a — Based on International Rating Scale with the following reaction ($R^{o}x$) categories.

2 — Resistant (0-15% infection)

5 — Intermediate (16-45% infection)

8 — Susceptible (46-100% infection)

^{b}20 — plants were used per variety

acute apices. Conidial length ranged from 32.0 to 61.2 μ and the width from 7.6 to 20.0 μ or an average of 46.72 x 12.28 μ (Table 1). In length 69% of the conidia measured fell within the limit of 42 to 52 μ while in width, 86% fell within the limit of 8 to 14 μ (Table 2).

The conidiophores were hyaline, erect, arising singly or in groups from stomata and dichotomously branched. The length ranged from 220.0 to 422.8 μ and the width from 9.2 to 22.0 μ, with an average of 325.25 x 15.22 μ (Table 1).

Virulence of species and reaction of different cane varieties

Table 3 shows the percent systemic infection and reaction of different cane varieties to three *Sclerospora* species 20 weeks after inoculation under field conditions. *S. philippinensis* was the most virulent with a mean systemic infection of 28.36%, followed by *S. sacchari* and *S. spontanea* with 14.04% and 12.07% systemic infection, respectively.

Badila and Phil 6607 showed resistant reaction to all species. Phil 6317 and Phil 6425 were resistant to *S. sacchari* and *S. spontanea* but intermediate to *S. philippinensis,* whereas Phil 58260 and Phil 6429 showed intermediate reaction to *S. sacchari* and *S. spontanea* and susceptible to *S. philippinensis.*

DISCUSSION

The ten *Sclerospora* isolates from sugarcane and from *Saccharum spontaneum* consisted of three distinct groups represented by *S. philippinensis, S. sacchari* and *S. spontanea* based on symptoms, morphological characteristics and reaction of different cane varieties. The first two were isolated from sugarcane and the last from *Saccharum spontaneum.*

The symptoms produced by each of the three *Sclerospora* species on standard test cane (Phil 58260) showed distinctive patterns which could serve as one of the bases for differentiating them into distinct species. *S. philippinensis* produced a pale yellow or whitish discoloration on the base of the 4th and 5th leaves after inoculation. The succeeding leaves showed a whitish discoloration all over the leaf blade without reddish discoloration on the affected tissues and leaf shredding. Cane plants infected with *S. Sacchari* exhibited greenish yellow streaks on the leaf blade which later on turned reddish and shredded as the leaves become old. This observation collaborated the findings of Elazegui and Exconde[5] who noted the same type of symptoms on sugarcane inoculated with *sacchari*. Some of the plants infected with this species were stunted and had abnormally thin stalks and narrow discolored leaves similar to those described by Hughes and Robinson[7] The symptoms produced on sugarcane inoculated with *S. spontanea* closely resembled those inoculated with *S. sacchari,* except for the presence of light yellow, irregular and discontinuous streaks on the leaf blades and absence of growth stunting.

The morphological characteristics of the three *Sclerospora* species on Phil 58260 cane differ significantly.

The shapes of conidia and conidiophores of *S. philippinensis* (Isolate 1) were similar to those of typical *Sclerospora philippinensis* on corn as described by several investigators (Weston[14]; Elazequi and Exconde[5]; Exconde[6] *et al;* and Visarathanonth and Exconde[13]). The conidia were spherical when young and oval elongate or ellipsoid when mature. The conidiophores were typically dichotomous, producing two primary branches on which two to four secondary branches terminated severally in one or two tapering sterigmate where the conidia were borne.

The sizes of conida and conidiophores varied. Weston[14] reported that conidia of *S. philippinensis* ranged from 27to39μ long by 17 to 21μ wide and conidiophores, 150 to 400μ long by 12 to 24μ wide. In the present study, the conidia of *S. philippinensis* ranged from 20.91 to 42.64μ long by 10.25 to 19.68μ wide and the conidiophores, 262.8 to 396.4μ long by 10.0 to 14.8μ wide. The control length difference between this species and *S. sacchari* or *S. spontanea* was highly significant. Width difference was observed between this species and *S. spontanea* but not with *S. sacchari.* Difference in conidiophores length between this species and *S. sacchari* was highly significant but not *S. spontanea.* Highly significant differences in conidiophores width were observed among species. The description and measurements of conidia and conidiophores of this species fit very closely those given by Weston[15], Elazequie and Exconde[5], Exconde[6] *et al.* and Visarathanonth and Exconde[13] for *S. philippinensis.*

The shapes of conidia and conidiophores of *S. sacchari* (Isolate 5) on sugarcane reported in this study were the same as those of typical *Sclerospora sacchari* Hiyake described by Hughes and Robinson[7]; Rivera[10]; Elazequi and Exconde[5]; and Visarathanonth and Exconde[13]. The conidia were elliptical or oblong with round apex and slightly spiculate base. The conidophores were characteristically dichotomous, with branching pattern similar to that of *S. philippinensis,* except that the branches were longer and slender sterigmata. The sizes of conidia of *S. sacchari* ranged from 31.16 to 52.89μ long by 9.43 to 19.27μ wide and conidiophores, 190.8 to 432.4μ long by 7.2 to 14.8μ wide. These measurements agree very closely with those reported by Elazequi and Exconde (1968) for *S. sacchari.* According to Hughes and Robinson (1961), Hiyake found in 1911 that the conidia of *S. Sacchari* measured from 25 to 41μ long by 15 to 23 μ wide and the conidiophores, from 160 to 170μ long by 10 to 15μ wide. The measurements of *S. sacchari* were within the range reported by these authors.

Conidia of *S. spontanea* were elongately ellipsoid or cylindrical with round to acute apicies, more slender and longer but narrower than those of *S. sacchari.* On sugarcane, the conidia measured ranged from 32.0 to 51.2μ long by 7.6 to 2.0μ wide and the conidipohores, 220.0 to 422.8μ long by 9 to 22μ wide. Those description and measurements of conidia and conidiophores are in very close agrement with the description and measurements given by Weston[15] and Visarathanonth and Exconde[13] for *S. spontanea.*

The sugarcane varieties used indicated differences in reaction to the species as shown by the percent infection after five months under field conditions. Of the three *Sclerospora* species, *S. philippinensis* was the most virulent, with a mean systemic infection of 28.26% on all test canes. *S. spontanea* was the least virulent,

with a mean systemic infection of 14.04%. A highly significant difference in percentage of systemic infection was obtained between *S. philippinensis* and of either *S. sacchari* or *S. spontanea.* The difference in percent systemic infection between *S. sacchari* and *S. spontanea* was insignificant.

Based on the rating scale used, *S. sacchari* and *S. spontanea* exhibited virulence pattern distinctly different from those of *S. philippinensis.* The above findings further revealed that *S. sacchari* which is commonly found attacking sugarcane in the Philippines was far less virulent than *S. sacchari* as reported by other workers abroad (Chu[2]; Edgerton[4]; and Hughes and Robinson[7]).

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ESPECIES DE *SCLEROSPORA* QUE CAUSAN LA ENFERMEDAD AÑUBLO LANOSO DE LA CAÑA DE AZUCAR (*SACCHARUM OFFICINARUM* L.) EN FILIPINAS

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RESUMEN

Se identificaron tres especies de *Sclerospora* de 12 aislados que se obtuvieron de la caña de azúcar afectadas por el y de *Saccharum Spontaneum* que fue recolectada de areas de siembra de caña de azucar tales como: – Negros Occidental, Pampanga, Tarlac y Laguna. A base de la sentomatologra y morphologia se identificaron tres especies de *Sclerospora: Sclerospora philippinensis, Weston, S. sacchari* Miyake y *Sclerospora spontanea* Weston.

Los sintomas caracteristicos producidos por cada una de las tres especies de *Scleropsora* en la caña de la prueba patron. (Phil 58260) son diferentes. *S. philippinensis* demostro descoloracion blanca solamente en la base de las hojas jovenes y hasta el punto de las hojas siguientes sin desmenuzamiento de las hojas, mientras *S. sacchari* produce rayas clocoticas bien definidas en las hojas con desmenuzamiento de las hojas caracteristicas y manismo de la planta afectada. Los sintomas manifestados por *S. spontanea* fueron los mismos que las de *S. sacchari,* con la excepcion de la presencia de rayas ligeramente amarillas, irregulares, y descontinuadas y la ausencia de emanismo de crecimiento.

Se observo la diferencia morfologica altamente significativa en

el largo y el tamaño de los conidioforas y en el largo de las conidias entre las tres especies de *Sclerospora.* La conidia de *S. philippinensis* fue alargada-oval o elipsoidal cuando la de *S. sacchari* fue eliptica u oblongada con el ápice redondeado y la base liguamente apiculada. *S. spontanea* varia liguamente de *S. sacchari* en la forma de la conidia. La conidia de *S. spontanea* fue más alargada elipsoidal o cilindrica con los ápices redondrados y agudos.

STUDIES ON THE EPIDEMIOLOGY OF SUGARCANE MOSAIC VIRUS

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ABSTRACT

The spread of sugarcane mosaic virus (SCMV) was found to be local in nature. The aphids have negligible role in its spread under natural conditions. Furthermore, it was proved beyond doubt that using a healthy material for planting was an efficient method for the control of SCMV. When aphids were tested for their virulence and efficiency in transmission under optimal condition by using local lesion assay host viz, soybean (*Glycine Max.* Merr.), it was found that the ability of aphids to transmit did not exceed 20% under such conditions which do not usually occur in the field.

MATERIALS AND METHODS

Healthy cuttings of Co. 419 variety were obtained from Cannanore, Kerala State, India where germplasm is being maintained at the Sugarcane Breeding Institute. The trial was initiated in February, 1977. The foci of infection were introduced at four levels: 5, 10, 25 percent and control. Each treatment was replicated five times. A plot contained five rows which were five feet long. The distance between two rows was 70 cm with eight plants planted in each row. Observations were recorded at 15 days interval to determine the number of infected plants. The pattern of spread was analyzed according to Vander Plank formula (1963), which is as follows:

$$\frac{\text{No. of infected plants} \times \text{No. of infected plants} - 1}{\text{Total No. of plants}}$$

The same experiments were repeated in March, 1978 with the establishment of a barrier crop which was supposed to be immune to SCMV but not to aphids. Ten feet strips of sunflowers were planted around the field on all directions to serve as barrier crop. The same number of treatments and the same number of replications as in the previous experiments were maintained.

After a month, the number of infected plants was taken at 15 days interval. The actual as well as the calculated number of doublets were recorded.

In order to find out the efficiency of aphids in the transmission of SCMV using soybean as local lesion assay host, the following experiment was conducted.

Healthy soybean seedlings, 10 to 12 days old were kept in the dark room for 24 hrs. after which aphids from colonies previously established on diseased sugarcane Co. 419 seedlings, were transferred to the cotyledonary leaves of soybean plants at the rate of one aphid per plant. The plants were kept again in the dark room for another 24 hrs. Then the plants were exposed to mercury vapor lamp illumination. Within 2-3 days, the translocated necrotic local lesions were clear enough to be counted.

RESULTS

The disease incidence also increased when the foci of infection as reflected by percentage in infected setts increased (Table 1). Maximum disease incidence was observed when the inoculum was at 25 per cent level. However, the disease incidence in plants with 10 per cent inoculum level was on par. The number of infected plants in plots with 5 percent inoculum level was significantly reduced. The control showed the lowest number of infected plants.

TABLE 1. Number of infected plants and analysis of variance (1977)

Treatment number	Foci percentage	Replications I	II	III	IV	V	Total	Mean
I	5	27	33	33	57	33	163	32.60
II	10	33	40	40	37	38	188	37.60
III	25	40	40	40	40	40	200	40.00
IV	0	22	30	30	29	30	141	28.20
Total		122	143	143	143	143	692	
Mean		30.5	35.8	35.8	35.8	35.3		

SE	*CD (P = 0.05)*		
1.33	*2.90*		
25%	*10%*	*5%*	*Control*
40.0	*37.6*	*32.6*	*28.2*

The number of infected plants in the different treatments in replication I was uniformly less than the number of infected plants in the corresponding treatments in other replications. Such might be due to the location of unplanted area in close proximity to first replication and the presence of sugarcane plants in all the three directions of the other replication. Presence of infected sugarcane

crop acting as an additional foci of infection might have favored rapid spread of the disease resulting in increase in the number of infected plants. The spread of SCMV could be considered to occur from the internal source of infection since the number of doublets actually observed was higher than the calculated number.

The same experiment was repeated in the succeeding year to confirm the results obtained in the previous season. The only difference was the inclusion of a barrier crop like the sunflower (*Helianthus annus L.*), around the experimental plot to study its effect on SCMV incidence. The observations were recorded at fortnightly intervals. The actual number of doublets observed was 25. The calculated number of doublets was as one. Therefore, the number of doublets actually observed was higher than the calculated number (Tables 2 and 3).

TABLE 2. Number of doublets observed in the field in 1977

Line No.	Replications I	II	III	IV	V	Total
1	14	14	19	16	15	78
2	14	11	19	15	15	74
3	9	13	14	15	16	67
4	6	17	19	17	17	76
5	15	19	19	19	14	86
6	11	19	14	17	18	79
7	19	15	9	14	12	69
8	19	17	17	19	14	86
Total	107	125	130	132	121	618

TABLE 3. Number of doublets observed in the field in 1978

Line No.	Replications I	II	III	IV	V	Total
1	2	4	0	1	0	7
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	1	0	3	1	0	5
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	2	3	1	4	3	13
Total	5	7	4	6	3	25

The above results confirmed the previous finding. In addition to that, the present study proved the efficiency of sunflower as a barrier crop, in

TABLE 4. Number of infected plants

Treatment No.	Foci percentage level	Replications I	II	III	IV	V	Total	Mean
I	5	1	0	1	0	0	2	0.40
II	10	1	2	2	2	2	9	1.80
III	25	3	4	3	4	3	17	3.40
IV	0	0	0	0	0	0	0	0.00
Total		5	6	6	6	6	28	
Mean		1.25	1.50	1.50	1.50	1.25		

SE 0.22 *CD* 0.68

eliminating the disease to negligible proportion. The present study did not show any role of the winged aphids in the spread of SCMV. This was confirmed by indexing the aphids on soybean as local lesion host (Table 4).

It can be concluded that sugarcane mosaic virus spreads from loci of infection and could be checked completely by carefully selecting healthy materials and establishing nurseries surrounded by sunflower as a barrier crop in our condition. This can be easily adopted by sugar factories.

TABLE 5. Efficiency of *Rophalsiphum maidis* Fitch in the transmission of SCMV as expressed by number of soybean plants showing translocated lesions

Replication No.	No. of soybean plants inoculated with aphids	No. of plants showing local lesions	Mean No. of lesions per plant	Percentage of plants infected
I	94	15	10.66	15.9
II	126	23	9.50	18.2
III	117	17	11.66	14.5
IV	110	19	10.50	17.2
V	120	18	12.00	15.0
Control	45	0	0.00	0.0

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ESTUDIOS SOBRE LA EPIDEMIOLOGIA DEL VIRUS DEL MOSAICO DE LA CAÑA DE AZUCAR

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RESUMEN

Se reveló que la dispersión del virus del mosaico de la caña de azucar es de naturaleza local y que los afidos no tienen importancia en la dispersion de la enfermedad bajo condiciones naturales. Además, se probo que el uso de material sano para la siembra es un metodo eficiente para controlar la enfermedad. Cuando los afidos se probaron (por su virulencia y eficacia en transmisión) bajo condiciones optimas para la actividad de los mismos, usando huéspedes de lesiones locales i.e. Soga (*Glycine Max.* Merr), se encontró que la habilidad de transmision de estos no sobrepaso el 20 porciento bajo unas condiciones óptimas y las cuales nunca estarán disponibles bajo condiciones de campo.

SUGARCANE MOSAIC VIRUS IN TAIWAN

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ABSTRACT

Five Taiwan isolates of sugarcane mosaic virus (SCMV) were classified on the basis of symptoms on differential host varieties. The SCMV strains from Taiwan were as follows: the fine stripe type and the necrotic type = mixture of SCMV-A and -B; the short stripe type = SCMV-B; the yellow stripe type = SCMV-A. and a Badila cane isolate = SCMV-D. The data obtained on physical properties, serology, purification, and electron microscopy were consistent with these identifications, although these methods could not be used independently, without symptoms, as the identifying criteria.

INTRODUCTION

In 1950, Liu[8] reported the presence of at least four strains of sugarcane mosaic virus (SCMV) in Taiwan. He listed a short stripe type, a necrotic type, a yellow stripe, type and a fine stripe type. These were collected on the commercial varieties of sugarcane present at that time. Sugarcane clones F 108, POJ 2883, Co 281, 35-119 and 34-1347 were found to be useful in strain differentiation. In 1953, Liu and Li[7] reported that only three strains (fine stripe, short stripe, and yellow stripe) were present in Taiwan; they mentioned no necrotic symptoms. Leu and Bau[6] later also found only the three distinct strains, but they mentioned that the short stripe type produced a severe mottling, necrosis, and stunting when inoculated onto clones 40-1311, 41-667, 53-363 and 53-383.

Abbott and Stokes[1] attempted to classify the Taiwan strains with the differential host system used by Summers *et al*[9] and updated by Abbott and Tippitt[2]. Abbot and Stokes identified the yellow stripe as SCMV strain A and suggested that the fine stripe might be SCMV-F.

In this paper, a description was done to the tests to classify five isolates of SCMV from Taiwan by symptomatology on differential varieties and by electron microscopy, serology, purification, and physical properties, so that the Taiwan strains may be related to those found in the USA and other cane-growing countries.

MATERIALS AND METHODS

The yellow stripe type (YST), fine stripe type (FST), and necrotic type (NT) mosaic isolates were collected on *Saccharum* interspecific hybrid 53-363 and the short stripe type (SST) was collected on hybrid 54-420. The FST, SST, and YST were thought to represent the types described by Leu and Bau[6]. The NT was the type obtained by inoculation of 53-363 with SST and maintained in the field since 1965 (Leu and Bau[6]). The Badila isolate (BdI) was collected on the *S. officinarum* clone Badila (a chewing cane). All isolates were collected in Taiwan.

Varieties with symptoms diagnostic for strain identification were used. These were *Saccharum* hybrids CP 31-294, CP 31-588, and Co 281; *Sorghum bicolor* cv. 'Rio'; and *Sorghum halepense* (Johnsongrass). These varieties were also inoculated with strain A, B, or D of SCMV. The Taiwan isolates were tested twice (in the fall of 1977 and the following winter). The *NT* and FST isolates were tested again in the spring of 1978. These tests were performed in the quarantine greenhouse at Beltsville, Maryland. Similar tests conducted in Taiwan during the winter and the spring did not have SCMV-A, -B, and -D available for comparison. Diagnostic varieties were inoculated with an artist's airbrush. Assay plants (Rio sorghum) used for thermal inactivation point (TIP) and dilution end point (DEP) tests were inoculated by the finger rub method. The source plants for the DEP tests were Rio sorghum and those for the TIP test, 'Sart' sorghum.

All of these isolates were purified from Rio sorghum by the method of Gillaspie[3]. Tissue was extracted by the ascorbic acid method, concentrated and purified by differential centrifugation followed by a rate-zonal centrifugation in sucrose density gradients. Samples for serology and electron microscopy were resuspended in 0.01 M borate buffer,. pH 8. Microprecipitin tests were used to compare the serological reactions of the Taiwan isolates to antisera of SCMV strains A, B, D, H, and I. Samples were negatively stained with 2% phosphotungstic acid, pH 7, for electron microscopy. Only the general morphology of the virus particles was determined and not accurate normal length.

RESULTS

All of the Taiwan isolates and the known SCMV strains produced infectious bands in comparable areas of sucrose density gradients after rate zonal centrigufation and all were flexuous rods resembling those of the potyvirus group. All of the Taiwan isolates and SCMV-A. -B, and -D had a TIP between 54 and 57 C. The DEP varied from 1:00 to 100,000 from one test to another, but all of the isolates, except NT, and the known strains had average DEP's of 1:1000 to 1:100,000. The NT isolate DEP was 1:10,000 to 1:100,000.

The diagnostic variety test results indicate that isolate YST was SCMV-A, Bdi was SCMV-D, and SST was SCMV-B. All of the isolates produced mild mosaic symptoms on Co 281 and did not infect johnsongrass, eliminating the possibility of SCMV-C or maize dwarf mosaic virus. Isolates FST and NT differed somewhat from the described strains. The FST and NT isolates produced some symptoms of SCMV-A and some of SCMV-B. Symptoms of an artificially produced mixture of SCMV-A and -B were similar to the symptoms of FST on CP 31-294 and CP 31-588.

The NT symptoms were more variable than those of the other isolates. Some CP 31-294 plants infected with NT had symptoms very similar to those of SCMV-B alone and other plants had symptoms similar to those of a SCMV-A and -B mixture. As the symptoms produced by NT differ from those of FST even though both isolates appear to be mixtures of SCMV-A and -B, it is probable that the components of the mixture are maintained in different ratios (Gillaspie and Koike[4]).

The microprecipitin test data, shown in Table 1, indicate that all of the Taiwan isolates are serologically related. All produced heterologous titers similar to or lower than the homologous titers with SCMV-A, -B, -D, and -H antisera and no reaction with SCMV-I antiserum. These results correlate well with the strain identification obtained from the differential variety tests described above.

TABLE 1. Classification of Taiwan isolates of sugarcane mosaic virus

Taiwan Symptom Designation	Sugarcane Variety Source	SCMV Strains [1]	Microprecipitin Reaction [2] Antiserum to: A, B, D	H	I
Fine Stripe type	53-363	A + B	++	+	–
Necrotic type	53-363	A + B	++	+	–
Short stripe type	54-420	B	++	+	–
Yellow Stripe type	53-363	A	++	+	–
Badila isolate	Badila	D	++	+	–

[1] *Classification of sugarcane mosaic strains according to Summers et al.*[9] *and Abbott and Tippett*[2].

[2] *Serological reactions: + + = titer about the same as homologous titer of antiserum: + = titer lower than homologous of antiserum – = no reaction.*

DISCUSSION

Symptomatology, purification, serology, physical properties, and electron microscopy data are consistent with the identification of the Taiwan isolates as follows: BdI = SCMV-D, FST and NT = mixtures of SCMV-A and -B, SST = SST = SCMV-B, and YST = SCMV-A.

Our DEP and TIP results for SCMV-A, -B, and -D agree closely with those of Abbott and Tippett[2], Tosic and Ford[10], and Zummo[11] except for the slightly lower TIP value obtained for SCMV-A (52-53 C) by Abbott and Tippett. This close agreement is gratifying because of the variety of source and assay plants used in obtaining these results by the different authors. The variation in TIP and DEP data among tests did not allow use of these data in strain identification.

There are indications that FST has taken on the characteristics of a new strain. Sugarcane clones 53-22, 53-29, and 53-32 are resistant to YST (SCMV-A) and SST (SCMV-B) but very susceptible to FST (SCMV-A and -B); and *Saccharum barberi* is susceptible to YST and SST, but immune to FST (Leu and Bau[6], Liu and Li[7]).

Our results for the YST isolate are similar to those of Abbott and Stokes[1], but they found FST to be similar to SCMV-F. Strain F could be included in the FST mixture that we tested since the mild symptoms of SCMV-F could probably be masked by the more severe symptoms of SCMV-A and -B. The FST and NT isolates reacted with antiserum to a Pakistan isolate (4975) that has been tentatively identified as SCMV-F (Gillaspie *et al.*[5]) and BdI, SST, and YST failed to react significantly in microprecipitin tests with the 4975 antiserum (R. W. Harris, Unpublished data). However, the 4975 antiserum was of low titer, so that the significance of these data is unknown. Also, FST does not infect johnsongrass, and the 4975 isolate does. The NT isolate used had apparently become contaminated during the 12 years it was maintained under field conditions since NT and SST had very different symptoms on the diagnostic sugarcane clones.

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LAS CEPAS DEL VIRUS MOSAICO DE LA CAÑA EN TAIWAN

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RESUMEN

Se identificaron cinco aislados del virus del mosaico de la caña de azucar de Taiwan a base de las sintomas en las variedades diferenciales. Las Cepas del virus mosaico de Taiwan fueron designadas como sigue: el tipo de raya fina y de necrotica de Taiwan = una mezcla de cepa A y B; el tipo raya corta = cepa B; el tipo raya amarilla = cepa A; y el aislado de la caña Badila = cepa D.

La data obtenida sobre la propiedad fisica, la serologia, la purificacion y la microscopia electronica fueron consistente con las identificaciones, aunque los metodos no se usan independientemente como criterios de identificaciones sin sintomas.

STRAINS OF THE SUGARCANE MOSAIC VIRUS IN THE PHILIPPINES

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ABSTRACT

Six sugarcane mosaic virus isolates collected from the different sugarcane-producing provinces of the country were used for strain identification. Symptomatology test revealed 3 strains were present in the collection as indicated by the differences in symptom pattern produced in the differential host. Serology demonstrated that virus isolates from Laguna and Tarlac reacted strongly to antiserum of strain A and isolates from Batangas, Pampanga and Negros Occidental were found serologically related to strain D. Leyte isolate produced heavy reaction with strain H but no reaction against other antisera. Physical properties were observed to be of limited value in differentiating SCMV strains. On the bases of symptomatology and serological reaction, strains A, D and H were definitely identified.

Strains A and D seem to be the most common strain with strain D producing the severe symptom among the three isolated strain.

INTRODUCTION

Ever since sugarcane mosaic was first reported present in the Philippines in 1920, little information about its causal factor were available. During this period and the succeeding decades, researches were concentrated mainly on monitoring its occurrence and distribution throughout the country. Limited studies were conducted because the disease has not been a serious problem in the sugarcane industry. However, in 1976, a high incidence of mosaic was reported not only in Negros province but also in some provinces of Luzon. This recurrence of an alarming mosaic infestation prompted efforts to study in depth the status of sugarcane mosaic virus. This paper is concerned primarily with strain identification of different virus isolates collected in selected provinces of the Philippines.

MATERIALS AND METHODS

Sources of Inoculum

A representative sample of mosaic-infested cane from the provinces of Negros Occidental, Leyte, Laguna, Batangas, Tarlac and Pampanga were collected and planted in the greenhouse to serve as inoculum source. No mosaic virus-infected canes were observed in other provinces at the time of collection. Each sample collected was labelled as one virus isolate for strain identification.

Preparation of Inoculum and Inoculation Technique

The inoculum was prepared through sap extraction by macerating bits of mosaic-infected leaves in a sterile mortar and pestle. The sap extract was homogenized in neutral borate buffer solution before it was inoculated in the differential host plants. Sugarcane differential hosts were inoculated when they were at the 2 to 3 leaf stage, following the technique of Fischer and Lockheart[3]. Inoculation was done by wrapping the sap-soaked cotton wad around the spindle of the host plants. An insect pin was thrust rapidly in and out repeatedly through the different parts of the sap-soaked cotton wad into the healthy spindle. The pricking was made for several times until the desired number of pricks were attained. Differential host plants that escaped infection were re-inoculated using the same technique.

Differential Host

Four sugarcane varieties, namely: CP 31-294, CP 31-588, CP 29-291 and CO 281, obtained from Philsucom, La Granja, were used as standard differential hosts. The hosts grown and maintained in the greenhouse were inoculated with infectious sap extract by means of a needle prick when they were at 2 to 3 leaf stage.

Three healthy seedlings per differential host were tested with each virus isolate collected. Another three seedlings for each host were used as control.

Symptomatology

Inoculated differential host plants were observed for symptoms at bi-weekly intervals for two months or more. The mosaic symptoms exhibited by these differential hosts were compared to all thirteen strains described by various workers paying special attention on the nature of chlorosis, whether mild mottling or severe pattern of chlorotic streaks or stripes and presence or absence of necrosis in the diseased leaves.

Serology

The test was conducted to further confirmed the result obtained from the symptomatology test. The procedure used by Whitecomb and Black[3] was followed

in this test. The antisera purchased from the American Type Culture Collection in Maryland, USA were tested against the different collection of SCMV antigen (purified virus) using the righ precipitin test in tube. A homologous and heterologous reaction were conducted against the purified antigen. This was done by placing each antiserum of known strain in the bottom of six glass tube to serve as underlayer. These tubes correspond to six isolates tested. A healthy serum (collected from the blood of a healthy rabbit) in 1% sucrose solution was also placed in another glass tube as underlayer to serve as control. The antigen was layered on top (overlayer) of the antiserum to form a clear interface with the underlayer. After layering the antigen, the tubes were allowed to stand for 2 to 4 hours before observation was made. Observations was taken in darkened room except for a beam of light on the interface.

Physical Properties

The physical properties of the different virus isolates were studied to determine the stability of the virus.

1. *Thermal Inactivation Point.* The test was undertaken by placing 2 ml of infectious sap each in 5 test tubes and heated in water bath with corresponding temperature of 40°, 50°, 60° and 70°C for ten minutes. The fifth tube was not heated to serve as control. After heating the tubes were immersed in cold water to cool off before the sap was inoculated in a weekly-healthy corn seedlings.

2. *Dilution End Point.* A 1:1 dilution was prepared from infectious sap extracted from 2 gm infected leaves with 2 ml of distilled water. From this sap preparation, a 1:10 dilution was prepared by diluting 1 ml of infectious sap with 9 ml of distilled water. From this dilution, a series transfer was made until a dilution of 1:10,000 was attained. Each dilution was then inoculated mechanically to one-week old corn seedlings.

3. *Longevity of Vitro.* The retention of infectivity at room temperature was determined on an aged borate buffer added sap extract. The extracts were stored in vials and samples were withdrawn at bi-hourly on the first day and daily thereafter for infectivity test. The daily inoculation were continued on a one-week old corn seedlings until the infectivity of the virus was lost.

RESULTS

Symptomatology

The result of the inoculation test is presented in Table 1. Except for Co 281, all the other differential hosts were infected by the different virus isolates.

The symptoms produced in the differential hosts were used as one of the criteria in differentiating the virus strains. For instance, the symptom produced by the isolates from Negros Occidental, Pampanga and Batangas begun as long

TABLE 1. Infection of sugarcane differential hosts with six isolates from SCMV from mechanical inoculation[a]

Isolates	Differential Host[b]			
	CP 31-294	CP 31-588	CP 29-291	CO 281
Batangas	+	–	–	–
Laguna	–	+	+	–
Leyte	+	–	+	–
Negros Occidental	+	+	–	–
Pampanga	+	+	+	–
Tarlac	–	–	+	–

[a] *Inoculation method – Pin prick at forty pricks/plant*

[b] *+ = With infection*

– = No infection

TABLE 2. Symptom description of six sugarcane mosaic virus isolates

Isolate	Differential Host			
	CP 31-294	CP 31-588	CP 29-921	CO 281
Batangas	Long, discrete chlorotic streaks through entire leaf blade	No infection	No infection	No infection
Laguna	No infection	Mild mosaic symptom; chlorosis not so pronounce	Ordinary mosaic	No infection
Leyte	Faint patter of mild mottling; symptom disappear; occasional death of tillers noted	No infection	Ordinary mosaic (faint) symptom	No infection
Negros Occidental	Long discrete chlorotic streaks through entire leaf blade	Chlorosis	No infection	No infection
Pampanga	Long distance chlorotic streaks through entire leaf blade	Chlorosis	Ordinary mosaic symptom	No infection
Tarlac	No infection	No infection	Ordinary mosaic symptom	No infection

severely chlorotic streaks (Table 2). Although the streaks were not continuous, they extended to the entire length of the leaf of CP 31-294. Both the Negros and Pampanga isolates produced a coarse pattern of severe chlorosis on CP 31-588, while the Laguna isolates produced a mild mosaic symptom on the same host.

Unlike the first four isolates described, the Leyte isolate exhibited a different type of symptom on CP 31-294. It produced a faint pattern of mild mottling. The symptom however, tend to disappear a few weeks after infection. Occasional death of the tillers were noted on the host infected by this isolate.

TABLE 3. Serological relationship among the different isolates of sugarcane mosaic virus (SCMV)

		Antisera (1/32)[a]				
Antigen	Dilution	SCMV-A	SCMV-B	SCMV-D	SCMV-H	Healthy Serum
Batangas	1/1	+	O	+++	/	
Isolate	1/32	/	O	++	–	–
	1/64	–	O	++	–	–
Laguna	1/1	++	–	–	O	–
Isolate	1/32	++	–	–	O	–
	1/64	+	–	–	O	–
Leyte	1/1	–	O	O	+++	–
Isolate	1/32	–	O	O	++	–
	1/64	–	O	O	/	–
Negros	1/1	–	–	+++	/	–
Isolate	1/32	–	–	++	–	–
	1/64	–	–	+	–	–
Pampanga	1/1	–	O	+++	/	–
Isolate	1/32	–	O	++	–	–
	1/64	–	O	+	–	–
Tarlac	1/1	++	–	O	–	–
Isolate	1/32	+	–	O	–	–
	1/64	+	–	O	–	–

[a] *O = No test conducted*
/ = Barely visible precipitate
– = No reaction
+ = Slight reaction
++ = Moderate reaction
+++ = Heavy reaction

Mild symptoms were produced in CP 29-291 by all inoculations of the different isolates. The Tarlac and Laguna isolates produced ordinary mosaic symptom (mild mottling with no necrosis) on this host.

Serology

The result of the serological test is presented in Tabie 3. Isolates obtained from Tarlac and Laguna reacted with SCMV strain A antiserum. Other isolates such as Negros Occidental, Pampanga and Batangas reacted with strain D antiserum, while the Leyte isolate reacted to SCMV strain H antiserum.

Physical Properties

1. *Dilution End Point.* The test showed that most of the virus isolates studied have similar tolerance to dilution except for the Tarlac isolate (Table 4). All the virus isolates were infectious from 1:10 to 1:1,000 dilution. The isolate from Tarlac was still infectious at dilution of 1:10,000 while the rest of the isolates produced negative result on this dilution.

TABLE 4. Dilution and point of the different isolates of SCMV

Isolate Source	Dilution[a]			
	1—10	1—100	1—1,000	1—10,000
Batangas	+	+	+	—
Laguna	+	+	+	—
Leyte	+	+	+	—
Negros Occidental	+	+	+	—
Tarlac	+	+	+	+

[a] *+ = With infection*
— = No infection

2. *Thermal Inactivation Point.* All isolates were able to withstand a temperature of 40°C (Table 5). Increasing the temperature to 50°C, all the isolates produced infection except for the Leyte isolate which failed to infect the corn test plants. At 60°C and 70°C all the virus isolates were inactivated.

3. *Longivity in Vitro.* It was observed that all the isolates remained infectious from 0-8 hours after extraction (Table 6). It was further noted that all the isolates were still infectious on the first day. On the second day, all isolates, except for Batangas isolate which retained its infectivity, lost their ability to infect after inoculation. Negative result was obtained for Batangas isolate on the third day.

TABLE 5. Resistance of different isolates of sugarcane mosaic virus to heat

Isolate Source	Temperature (°C)			
	40	50	60	70
Batangas	+	+	–	–
Laguna	+	+	–	–
Leyte	+	–	–	–
Negros Occidental	+	+	–	–
Pampanga	+	+	–	–
Tarlac	+	+	–	–

[a] *+ = With infection*
– = No infection

TABLE 6. Longevity of different sugarcane mosaic virus isolates in vitro at room temperature (28°C) on a daily basis

Isolate Source	Days[a]			
	0-8hr	1	2	3
Batangas	+	+	+	–
Laguna	+	+	–	–
Leyte	+	+	–	–
Negros Occidental	+	+	–	–
Pampanga	+	+	–	–
Tarlac	+	+	–	–

[a] *+ = With infection*
– = No infection

DISCUSSION

Identification of SCMV strains by symptomatology through differential hosts and serological reaction were more reliable than by physical properties study.

Using sugarcane varieties CP 31-294, CP 31-588, CP 29-291 and Co 281, it revealed that at least three strains existed. These were strain A, D and H.

Laguna and Tarlac isolates were found to be strain A. This conclusion was attained due to the symptom exhibited by CP 31-588 which is similar to those described by Abbott and Tippett. This claim was further substantiated by the reaction of the antigen of this virus to the reference antiserum (Strain A) but which no reaction was noted when the same antigen was tested against strain B, D and H antisera.

Batangas and Pampanga isolates together with that taken from Negros Occidental were identified as strain D. This finding was proven through the symptom produced by these isolates on CP 31-294 and CP 31-588 which is approximately the same type of symptom for strain D described by Abbott and Tippett and Tippett and Abbott[7]. The same finding was further asserted by the positive serological reaction of the antigen of these isolates of the reference antiserum for strain D.

On the other hand, the isolates from Leyte was identified as strain H. This discovery parallels the result of Abbott and Tippett[1]. They claim that strain H produced a faint pattern of mild mottling on CP 31-294, which often disappeared. This phenomenon was also observed in the experiment. Serologically, the identified strain H did not produce heterologous reaction against other antisera. This supports the findings of Snazelle *et al*[5]. They stated that strain H reacted only homologously and no heterologous reaction was obtained in other SCMV strains. Similar result was noted in the experiment.

Unlike the other differential hosts which were infected by the virus isolates, negative infection was obtained on Co 281. Because of this result, it is possible that the strains that could be differentiated by this host were not present among the six isolates tested.

The use of physical properties as a parameter, although essential in separating strains with similar symptom patterns was observed to be of limited value in differentiating SCMV strains in this study. A good basis to support this allegation for instance is the dilution end point of the virus, wherein all the isolates remained infective at dilution of 1:1,000. The result collaborated the finding of Adsuar[2] on the dilution end point. He reported that the DEP of SCMV is 1:1,000. The same DEP was obtained by Abbott and Tippett[1] for strain A and D.

On the other hand, the data obtained from thermal inactivation point indicated that majority of the isolates can tolerate only a temperature of up to 50°C. This agrees with the reports of Abbott and Tippett[1] that strain D and H were inactivated at temperature between 51°C to 53°C.

It seems that the result obtained from the reaction of infectivity of the virus negated the finding of Adsuar[2]. While he stated that sugarcane mosaic virus has a longevity of less than 7 hours, the result indicated that the isolated virus strains remained infectious from 1 to 2 days, very similar to those of Perone[4] and Teakle and Grylls[6] who showed that *longevity in vitro* of 1 to 2 days depends on the strains.

SUMMARY AND CONCLUSIONS

The identification and differentiation of sugarcane mosaic virus strains collected in some sugarcane-producing provinces of the Philippines was carried out by using different criteria such as symptomatology, serology and physical properties studies.

The nature of mosaic pattern on differential hosts and serological reaction were the principal diagnostic characters adopted in the study. On these basis strains, A, D and H were definitely identified.

It is very likely that other strains of SCMV, aside from the three strains already identified in the study are present in the Philippines. Further studies to determine the occurrence of other strains in other sugarcane-growing regions of the country are therefore recommended.

At the moment, symptom expression of the differential hosts and serological reactions seem to be the best criteria for differentiating and identifying strains of sugarcane mosaic virus.

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LAS CEPAS DEL VIRUS DE MOSAICO DE LA CAÑA DE AZUCAR EN FILIPINAS

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RESUMEN

Tres cepas del virus del mosaico de la caña fueron identificadas en Filipinas mediante la sintomalogia producida por estos en los huespedes diferenciales y sus reacciones a pruebas serólogicas. Las cepas identificadas fueron las cepas A, D y H. De estas la A y D son las cepas mas comunes y la D la de síntomas más severos.

INDUCTION OF SUGARCANE MOSAIC VIRUS VARIANT THROUGH HOT WATER TREATMENT

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ABSTRACT

A sugarcane mosaic virus variant designated as M, was obtained when diseased setts were treated by hot water at 52-C for one hour. Its symptoms consist of pale yellow short lines with deep green background. This variant also exhibited itself in sorghum varieties LCVCO 18, AS219, As 4482 and Atlas.

INTRODUCTION

The present study was carried out to induce mutation among SCMV strains with the object of producing a variant which produces very mild symptoms in order to reduce the loss caused by SCMV.

MATERIALS AND METHODS

In an attempt to obtain variants of SCMV, hot water treatment of infected sugarcane variety CO 419 at different temperature was performed. Infected canes were cut into three eye pieces and were treated in hot water bath at 50, 51, 52, 53, 54 and 55°C for one hr. Each treatment with three replicates consisted of 453 eyed pieces. The control was maintained along with the treatment. Immediately after hot water treatment, the cuttings were dipped in cold water for 1-2 min. and dried. All the treated pieces and control were sown in 6" pots and maintained under insect-proof glass house. Three to 4 weeks after germination the development of symptoms was observed.

Those seedlings that showed very mild symptoms compared to untreated cuttings were kept separately for one year. During this period, it was checked on *Sorghum bicolor* L. CV CO 18 which was used as a differential host for all strains. It was also tested on atlas sorghum, Rio, IS 9606, AS-219 *(S. cernum)* and AS-4482 and with great difficulty on CV Co. 18. It was then multiplied separately to check the stability of this variant. It was maintained after four serial transmissions on CV Co 18. This variant was designated as M_1.

RESULTS

When the sets were treated in hot water bath at 52°C for one hour, one seedling showed very faint symptoms in the early stages and the leaves produced subsequently showed no visible symptoms. The virus present in this seedling was multiplied and designated as variant M_1. This variant M_1 might have been produced either by selection from a mixture of strains which were sensitive to higher temperature or by mutation of the parent strain.

Symptoms induced by Variant M_1 on different hosts

1. Sugar CV Co 449 Pale yellow short lines with deep green background were formed. Canes beyond 3-4 months of age exhibited the symptoms clearly. When the canes infected with variant M_1 were ratooned, similar symptoms were observed. No necrosis or any further development of symptoms could be seen even during winter time when the temperature fell below 20°C.

During the same period necrosis was clearly observed on CO 419 and other varieties infected by other strains. The variations observed in the symptoms induced by variant M_1 remained constant through several sub-cultures. This definitely indicated that this character of mild symptoms induction was reliable attribute of variant M_1 in diagnosis.

2. Sorghum bicolor LCVCO 18: The infected leaves showed short pale yellow whitish lines covering the entire leaf on pale background. Green patches were found scattered interrupting the white lines.

3. *S. bicolor* L. (Rio): No visible symptom was produced.

4. *S. bicolor* L. AS-219 *(S. cernum):* The variant M_1 caused mosaic pattern throughout the leaf. This cultivar did not give any reaction when inoculated with other strains. It can be used as an indicator host for the variant M_1.

5. *S. bicolor* L. AS-4482 *(S. milforme):* Necrosis of leaves and stunting of plants were observed.

6. *S. bicolor* L. (Atlas sorghum): Mosaic symptoms alone were observed.

DISCUSSION

The variant M_1 obtained in the study might have been produced either by selection from mixture of strains which were sensitive to higher temperature or by mutation of the parent strain. This coincides with Abbott and Tippetts[1] observations that the strains of mosaic are slow to change and the temperature had no apparent effect on symptom expression or strain stability. Benda[2] stated that it is possible to find isolates of strain "A" and "B" which are identical to those of 30 years ago and that proved the independent identity of variant M_1

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ESTUDIOS SOBRE EL VIRUS DE LA CAÑA DE AZUCAR

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RESUMEN

Se logró induccion de un variante de las cepas del virus de la caña de azucar con agua caliente a 52° C por una hora. Los sintomas de este variante son muy ligeros y le designó como variante M.

EVIDENCE OF RESISTANCE IN *SACCHARUM SPONTANEUM* AND *SACCHARUM*-RELATED GENERA TO SUGARCANE MOSAIC VIRUS STRAINS H AND I

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ABSTRACT

Forty-five clones of *Saccharum spontaneum* and *Saccharum*-related genera were inoculated with a mixture of sugarcane mosaic virus (SCMV) strains H and I, the predominant SCMV strains in Louisiana's commercial sugarcane fields. Twenty-nine clones of different genera were found to be resistant to mosaic infection as evidenced by the lack of mosaic symptoms in the inoculated host plants, as well as in the highly susceptible 'Rio' sorghum assay plants which were inoculated with leaf extracts from the symptomless host plants. The resistant clones may be useful in basic sugarcane breeding for mosaic resistance.

INTRODUCTION

For most of this century, sugarcane mosaic has been a chronic disease problem for Louisiana's sugar industry (Abbott and Tippett[3], Breaux[4] and Koike[6], Summers *et al*[8]). Although there have been brief periods when mosaic incidence in commercial fields has been low due to the release and cultivation of cultivars resistant to the then prevalent sugarcane mosaic virus (SCMV) strains, the emergence of new SCMV strains has perpetuated the problem (Abbott[1,2]; Abbott and Tippett[3]; Koike[6]; Summers *et al*[8], Tippett and and Abbott[9]).

Breeding for mosaic resistance recently resulted in the production of a commercial cultivar, L 65-69, with high resistance to mosaic infection (Breaux and Koike[4]). Progenies of cv. L 65-69 contained several promising commercial-type cultivars with high resistance to mosaic infection. Nevertheless, there is a critical need for the identification of mosaic resistance in as large a gene pool as possible for use in developing mosaic-resistant commercial cultivars. Many of Louisiana's commercial sugarcane fields are heavily infected with mosaic (Koike[7]).

Tests were conducted to survey clones of *Saccharum spontaneum* and *Saccharum*-related genera to identify clones which are resistant to SCMV strains H

and I, the dominant strains in Louisiana's sugarcane fields (Abbott and Tippett[3] Koike[6]). Results of the tests are reported here.

MATERIALS AND METHODS

Forty-five clones of *Saccharum spontaneum* and *Saccharum*-related genera were tested. These clones were from the World Collection of Sugarcane Varieties, Canal Point (now Miami), Florida.

Six to 12 stools of each clone were transplanted separately into 10 cm day pots containing steam-sterilized soil. After several young shoots had developed, they were inoculated with a mixture of SCMV strains H and I.

The inoculum was prepared from young plants of *Sorghum bicolor* (L.) Moench cv. 'Rio'. The leaves infected with either SCMV-H or -I were ground in a mortar containing five parts (vol.) of 0.01 N sodium sulfite solution to one part (wt.) of leaf tissue and silicon carbide particles. After filtering through cheesecloth, equal volumes of the SCHV-H and -I extracts were mixed. Inoculation of the plants with the mixture was by the air-brush method. The shoots were inoculated three times at 9- to 10-day intervals.

The plants were examined for mosaic symptoms at weekly intervals for 4 weeks after the last inoculation. At the end of this period, all clones were assayed. The leaves were ground and extracted as described. Young 'Rio' assay plants in the 2-leaf stage were inoculated with the extracts (20-28 plants/extract), using thumb and forefinger on silicon-dusted leaves.

The test plants whose extracts did not produce mosaic symptoms on 'Rio' were again air-brush inoculated with SCMV-H and -I mixture; extracts from these test plants were assayed again on 'Rio' 4 weeks later as described earlier.

The experiments were conducted in a screened (30 mesh) greenhouse in 1978. The greenhouse was fumigated weekly, alternately with nicotine sulfate or tetraethyl pyrophosphate. The plants were fertilized at weekly intervals with a dilute solution of ammonium nitrate. Greenhouse temperatures ranged between 21° and 35°C.

RESULTS AND DISCUSSION

Leaf extracts from all clones exhibiting mosaic leaf symptoms produced mosaic in 'Rio' sorghum. The reaction to mosaic in clones of *Saccharum spontaneum* and *Saccharum*-related genera is summarized as follows:

Resistant – No mosaic symptoms in test plants nor in assay Rio plants.

1. *Saccharum spontaneum* clones: Djatiroto, Mandalay, SES 6, SES 114, SES 189, SES 196, SES 205B, SES 208A, SES 231, SES 304,

SES 577, Soembawa, Tainan 2n = 96, Taiwan 41, UM 70-003, US 56-15-2, US 56-15-8, US 56-16-1, US 59-1-1, US 61-2-1.

2. *Ripidium bengalense* (Imp. 2888), *R. procerum* (Imp. 3388).
3. *Miscanthus sinensis* (Imp. 3055), *M. sacchariflorus* (US 64-2-2), *Miscanthus* sp.
4. *Erianthus elephantinus* (SES 273), *E. bengalense* (US 57-10-2).
5. *Sclerostachya* sp. (US 56-5-5).
6. *Imperata* sp. (US 47-15).

Susceptible:

1. No mosaic symptoms on test plants, but symptoms on assay Rio plants.
   a. *S. spontaneum* clones: SES 50, SES 147B, SES 205A. Taiwan 2.
2. Mosaic symptoms on both test plants and assay Rio plants.
   a. *S. spontaneum:* SES 84/58, SES 278, SES 343, SES 351, SES 521, SH 246, Taiwan 34, US 60-4-6.
   b. *Ripidium procerum* (Imp. 1251), *R. kanashiroi* (Imp. 1690).
   c. *Miscanthus sinensis.*
   d. *Sclerostachya fusca.*

The symptoms on 'Rio' suggested the presence of both SCMV-H and -1 in the test plants. Extracts from symptomless test plants usually did not infect "Rio', indicating that these clones were not infected. Extracts from two of the symptomless clones (*S. spontaneum* clones SES 205A and Taiwan 2) produced distinct mosaic on 'Rio' plants, indicating that these plants were infected, although symptomless. Leaf extracts from two additional clones without typical symptoms produced mosaic symptoms on 'Rio' (Table 1); these were *S. spontaneum* clones SES 50 with reddish discoloration on the older leaves and SES 147B whitish streaks on a few leaves on young shoots.

Extracts from 29 clones without mosaic symptoms did not produce mosaic symptoms on 'Rio'. These clones were of *S. spontaneum* (20 of 32), *Ripidium* (2 of 4), *Miscanthus* (3 of 4), *Erianthus* (2 of 2), *Sclerostachya* (1 of 2), and *Imperata* (1 of 1). These clones could be valuable sources of genes for mosaic resistance that could be incorporated into commercial sugarcane cultivars.

In earlier tests in Louisiana, clones that remained symptomless after mechanical inoculation with mosaic were assumed to be resistant (Duncklеman and *Breaux*[5]). The results reported here emphasize the importance of assaying such symptomless plants on a highly susceptible host.

Results of these tests indicate that mosaic susceptibility does not follow

clear taxonomic relationships and each prospective parental clone must be tested individually.

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LA EVIDENCIA DE RESISTENCIA A LAS CEPAS H Y I DEL VIRUS DEL MOSAICO DE LA CAÑA DE AZUCAR EN *SACCHARUM SPONTANEUM* Y EN LOS GENEROS RELACIONADOS CON *SACCHARUM*

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RESUMEN

Se inocularon cuarenta y cinco clones de *Saccharum spontaneum* y géneros con *Saccharum* con una mezcla de las cepas predominantes H y I del virus del mosaico de la caña de azúcar en los campos comer-

ciales en Louisiana. Se encontró resistencia a la infección del mosaico en 29 clones de los diferentes géneros por la evidencia de ausencia de las sintomas del mosaico, tanto en las plantas huésped inoculadas como en las plantas altamente susceptible Rio sorgo, que fueron inoculadas con extracto de las hojas de las plantas huésped sin sintomas. Los clones resistentes puedan ser útiles en la hibridización básica de la caña para la resistencia al mosaico.

SUGARCANE MOSAIC AND YIELD OF SINGLE PLANTS OF SUGARCANE

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ABSTRACT

The effect of sugarcane mosaic on the yield of single plants of CP 65-357 was measured. Young cane plants with or without mosaic were transplanted in the spring from greenhouse to field and manually harvested in the fall. A plot consisted of a single plant on standard ridged row, with an intrarow distance of 183 cm, and an intrarow distance ranging from 19 to 152 cm. The plots of different treatments and replications were arranged as a Latin Square or as a complete randomized block experiment. The results indicate that the significantly higher sugar yield of healthy plants compared to mosaic-infected plants may be offset by increased recovery from infection, increased incidence of mosaic in the control and closer intrarow spacings of the diseased plants.

INTRODUCTION

In Louisiana, sugarcane mosaic occurs in epiphytotic proportions. To measure the effect of mosaic on the yield of a variety, replicated field-plot experiments are used, and these experiments are continued for some years in several locations. The results tend to be variable, and a variety's rating in terms of loss of yield (even in such broad terms as tolerant, resistant or susceptible) may vary among experiments. Factors known to contribute to this variability include the incidence of mosaic (Tippett[7]), the strain of virus and the recovery from infection (Tippett and Koike[8]), superinfection with ratoon stunting disease (RSD) (Koike[3]), and the stand of cane (Steib and Chilton[6]).

The variable results of the field tests indicate the need for a simpler and more incisive experimental technique to measure mosaic effects. One purpose of the experiments here reported was to test an alternative technique in which measurements on single plants were used to evaluate the factors that influence the yield of mosaic-diseased plants. In this study, the unit by which the response to mosaic is measured is the stalks of a single plant and not the population of stalks in the plot as in the field-plot technique.

MATERIALS AND METHODS

Field-grown, mosaic-free stalks of the variety CP 65-357 were selected; the stalks were progeny of cane heat-treated for the control of RSD. The stalks were divided in December or January into one-node seed pieces (cuttings) 12.5 cm long, of which 10 cm were above the leaf scar. These cuttings were planted vertically in 7.5 cm-diameter peat pots filled with vermiculite or soil, the pots with cuttings were placed in heated greenhouses for germination and early growth. As the young shoots grew, the leaves were cut back to promote thrifty plants. Leaves also were trimmed shortly before transplanting to the field.

To obtain infected plants, cuttings or young shoots were inoculated. Healthy controls were obtained from uninoculated cuttings from the same seed-cane stalk. For inoculation with the agent of RSD, the seed pieces were dipped in juice from infected stalks immediate after cutting. For mosaic, young shoots from cuttings were inoculated by the airbrush method (Dean[1]) with juice prepared from the spindle leaves of sugarcane plants infected with the appropriate virus strain. The strains of sugarcane mosaic virus used were A, B, D, H and I (SCMV-A, B, D, H, and I).

The young plants were transplanted to the field in March or early April. The fields of silty clay loam had been prepared for planting as is customary in Louisiana. The plants were transplanted into ridged rows so that the top of the seed piece was at soil level. The intrarow distance was 183 cm in all experiments; the intrarow distance between plants was 152 cm unless the effect of intrarow distance was to be evaluated. When the intrarow distance was a variable, each experimental plant was separated from adjacent plants by the designated distance; a healthy buffer plant was planted between experimental plant whenever the intrarow distance was to be changed. The intrarow distances between plants were 19, 38. 57, 65, 114 and 152 cm. The transplants were watered as needed until the first sufficient rain.

The experimental designs most used for planting in these experiments were a 6 x 6 Latin Square in which six treatments were each replicated six times or a complete randomized block in which each treatment was replicated four or five times. A split-plot design with main plots arranged according to a 6 x 6 Latin Square and subplots arranged according to complete randomized blocks was also used (Yates[9]). Each experimental plant was equivalent to a plot in all designs.

The plants were fertilized with nitrogen as anhydrous ammonia (about 135 kg/ha) according to plantation practice. When the plants were small, the sides of the row and the water furrows were cultivated repeatedly with discs fitted with guards to prevent soil from being added to the top of the row and changing the depth of planting. Control of the sugarcane borer larvae was by ground or aerial application of insecticides. Weeds were controlled by frequent hoeing.

In November, each experimental plant was harvested; all stalks 1.5 m and

over from soil level to top visible dewlap were manually cut after the tops had been examined for mosaic symptoms; the spindle and all leaves were removed. The stalks of the experimental plants were counted (millable stalk number), weighed (plot weight), and analyzed by standard methods for juice sucrose. The calculated juice sucrose (Legendre[5]) was converted to sugar % cane. The sugar (kg) per plant was calculated by multiplying plot weight by sugar % cane and dividing by 100. For convenience, the data based on the stalks of a test plant will be referred to as data per plant.

After the first harvest, the stubble was treated like plantation cane except that the stubble crop was manually harvested as the plant canes were.

The statistical analyses were appropriate to the design and missing values were so calculated. The significance of differences among means was determined using Duncan's multiple range test (Duncan[2]).

RESULTS

The results of these experiments indicate that the reduction in yield associated with mosaic was due primarily to reduced stalk number and to reduced stalk weight; differences in the content of sugar among mosaic-diseased and control plants often were not significant. The yield data nevertheless are reported in terms of sugar per plant which is the more inclusive parameter.

The cane apparently did not recover from infection with the isolates of SCMV-H or SCMV-I used in these experiments.

The Effect of Strains of the Sugarcane Mosaic Virus

The effect that strains of mosaic have on yield was compared in an experiment in which all plants were infected with RSD. As these plants were to be harvested repeatedly, the risk of uncontrolled infection with RSD seemed unnecessarily high but easily avoided by inoculation of all cuttings. In the plant-cane year, the yield from the plants infected with mosaic was less than that from plants free of mosaic (Table 1). The yield differences among plants infected with the various strains of mosaic were not significant. In the first-stubble crop, the situation became more complicated; mosaic virus (strain not determined) had begun to spread into the control, and recovery from mosaic infection occurred among plants previously infected with strains A, B, or D. The incidence of mosaic was determined by counting the number of millable stalks and noting for each stalk whether it had mosaic symptoms. Some stalks had tops that were too heavily damaged by borer or pokkah boeng to distinguish symptoms. These stalks were responsible for the difference between the incidence noted and 100% in plants infected with strains H or I. In the first stubble, the yield from control plants was not significantly different from that of plants originally infected with strains A or D, but the yield was significantly higher than that from plants originally infected with B, H, or I.

Significant differences in yield between mosaic-free and mosaic-infected plants had also been obtained in a 6 x 6 Latin Square experiment planted in 1971

in which the cuttings have been prepared from field-run cane. This experiment also showed no significant difference in yield among the mosaic-infected plants regardless of which one of the five mosaic strains was involved. No first-stubble data were obtained from this experiment in 1972 because of excessive lodging.

TABLE 1. The average yield (sugar/plant) as a function of the sugarcane mosaic virus strain and the mosaic incidence. (Intrarow distance between plants: 152 cm)

Inoculation	Plant cane (1973)		First stubble (1974)	
	Yield (kg)	Incidence (%)	Yield (kg)	Incidence (%)
SCMV-A	1.5 b[1/]	99	2.1 ab	31
SCMV-B	1.3 b	97	1.6 bcd	34
SCMV-D	1.5 b	100	1.8 abc	16
SCMV-H	1.2 b	100	0.9 d	93
SCMV-I[2]	1.4 b	98	1.2 cd	91
None	2.5 a	0	2.5 a	22

[1]*Average for six plants in a Latin Square design. Means followed by the same letter (read vertically) are not significantly different from each other at 5% level of probability.*

[2]*One missing plot*

The Effect of Both RSD and Mosaic

The yield from plants with RSD with or without mosaic, was compared with that from RSD free plants with or without mosaic. The strains of mosaic used were H and I. The results (Table 2) indicate that the yields in terms of weight and sugar

TABLE 2. The average yield in plant cane as affected by sugarcane mosaic virus strain (SCMV) and ratoon stunting disease (RSD). (Intrarow distance between plants: 152 cm)

Inoculation: Agent of	Avg. Weight/plant (kg)	Sugar % cane	Avg. Sugar/plant (kg)
SCMV-H	14.1 c[1]	12.1 c	1.7 c
SCMV-I	13.7 c	12.6 b	1.7 c
SCMV-H; RSD	10.1 d	12.6 b	1.3 d
SCMV-I; RSD	9.1 d	13.2 a	1.2 d
RSD	17.3 b	12.4 bc	2.1 b
None	21.1 a	12.1 c	2.6 a

[1]*Average of six plants in a Latin Square design (1977). Means followed by the same letter (read vertically) are not siginificantly different from each other at the 5% level of probability.*

per plant were highest among plants free of mosaic and RSD and significantly less among plants with RSD only. The yields were intermediate among plants free of RSD but with mosaic. The yields were least among plants with both RSD and mosaic. The sugar content (sugar % cane) of control and H-infected plants were significantly lower than that of the other treatments except the RSD-infected plants.

Stand of Cane Disease and Yield

The intrarow distance between plants was varied so that the yield per plant as a function of stand could be compared. In this series of experiments, the strain of mosaic was H. Only plant cane data were collected because at the closer spacing, the separate stools became indistinguishable in stubble.

The results (Table 3) indicate that sugar per plant increases as the intrarow distance between plants increases. The same trend was observed in each experiment whether plants were with or without mosaic or with or without RSD. This increase, however, is not evident at the closest spacings. A doubling of the intrarow distance from 19 cm to 38 cm increased the average yield per plant minimally or not at all. The values of the regression coefficient indicate that for each centimeter that the intrarow distance increases, the sugar per plant increases by 5 to 9 grams. As shown in Table 3, when the intrarow spacing was increased eightfold, the yield per plant increased only fivefold.

TABLE 3. The average yield of sugar per plant as affected by the distance between plants on a row.

Year	Inoculations: Agents of	Exper. Design	Replic.	Average kg. sugar/plant with intrarow distance between plants (cm)						Regress. coeff. (g/cm)
				19	38	57	76	114	152	
1975	SCMV-H	LS[1]	6	0.3 a[2]	0.4 a	0.4 a	0.6 ab	0.8 bc[3]	1.0 c	5.2
1976	SCMV-H	RB	5	0.3 a	0.4 a	0.6 ab	1.0 bc	0.9 b	1.4 c	7.5
1976	SCMV-H; RSD	RB	4	0.5 a	0.5 a	0.9 b	0.9 b	1.3 c	1.5 c	7.5
1976	RSD	RB	5	0.8 a	0.8 a	1.2 b	1.2 b	1.5 b	1.9 c	8.6
1976	None	LS	6	0.9 a	1.1 a	0.9 a	1.3 b	1.7 c	2 0 c	8.6

[1] *LS = Latin Square; RB = Randomized Block*

[2] *Means followed by the same letter — read horizontally — are not significantly different at 5% level of probability*

[3] *One missing value*

The role of the neighboring plant in spacing experiments was investigated in a split-plot experiment in 1977. The main plots were intrarow spacing arranged in a Latin Square while the subplots consisted of a healthy, an RSD-infected, and a mosaic-infected (SCMV-H) plant arranged in a randomized block design. The ana-

lysis of variance indicated that the interaction between distance and disease was significant. The results were calculated for each distance as complete randomized blocks (Table 4). They showed that mosaic-infected plants yielded significantly less than control plants at all spacings except at 38-cm. The yield from RSD-infected plants, with the exception of the 76-cm spacing, was not significantly less than that of the control plants. One mosaic-infected plant at the 19-cm spacing and one of the RSD-infected plants at the 38-cm spacing died long after they had become established. These plants were considered to have yielded zero.

TABLE 4. The average yield (kg sugar/plant) in plant cane as affected by intrarow distance and the health of plants

Intrarow distance (cm)	Plants inoculated with agent of Mosaic		
	Control plants	RSD	(SCMV-H)
19	0.7 a[1]	0.4 ab	0.2 b[2]
38	0.8 a	0.5 a[2]	0.6 a
57	1.2 a	1.1 ab	0.8 b
76	1.6 a	1.2 b	0.8 c
114	1.9 a	1.7 a	1.0 b
152	2.2 a	1.6 ab	1.2 b

[1]*Average for six plants in a split-plot experiment (1977). Means followed by the same letter read horizontally are not significantly different from each other at the 5% level of probability.*

DISCUSSION

The results with single sugarcane plants as the experimental until indicate that differences in yield between plants with and without mosaic were readily detectable. The magnitude of the differences, as had been found in replicated field-plot experiments (Koike[3], Steib and Chilton[6], Tippet[7] and Tippet and Koike[8]) is affected by the incidence of mosaic and the occurrence of recovery (Table 1), by superinfection with RSD (Table 2) and by the stand as indicated by the distance between plants (Table 4).

The magnitude of the differences in yield between healthy and mosaic-diseased plants was dependent on the interaction with cane stand. The difference in yield so evident at wider spacings (Table 1, 2, 4) may become small or not significant at close spacings, e.g., the result for the 38-cm spacing (Table 4). This convergence of the differences in yield may depend on the capacity of mosaic-diseased plants to develop millable stalks. This capacity is obviously limited compared to healthy plants at wide spacings where each plant can have many stalks. It may however, be sufficient at close spacings where each plant, healthy or diseased, can only have a few stalks. Further experiments are needed to determine the stalk number and stalk weight of mosaic-diseased plants at close spacings without the direct competition from healthy plants.

A second effect of close spacing, both for mosaic-free and mosaic-diseased plants, is to increase the yield per plant per unit area. This follows from the data of Table 3. As the intrarow distance increased, the yield per plant increased less than the spacing per plant. The yield per unit area increased especially at the closest intrarow spacing (from 38 to 19 cm). This increase was also observed in other experiments with healthy plants of CP 65-3574.

The use of single plants transplanted to the field in a replicated yield experiment can be useful to determine some effects of the disease. It offers greater precision at less expense than the standard field experiments and allows for more typical growth and yield than the greenhouse yield experiments. However, it cannot be used to measure the effects of disease on germination nor the effects of early spread of a disease.

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EL MOSAICO DE LA CAÑA DE AZUCAR Y EL RENDIMIENTO DE LAS PLANTAS INDIVIDUALES DE LA CAÑA DE AZUCAR

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RESUMEN

Se determinó el efecto del mosaico de la caña de azucar en el rendimiento de las plantas individuales de la variedad CP 65-357. Se transplantaron las plantas de la caña joven con o sin mosaico desde el invernadero a campo en la primavera y se cosecharon a mano en el otoño. Una parcela consistia de una planta individual en una hilera normal caballetada con una distancia entre hilera de 183 cm y una distancia en la hilera variando desde 19 a 152 cm. Las parcelas de los diferentes tratamientos y las replicaciones se arreglaron en un diseño de cuadrado latino o en un experimento de bloques completamente al azar. Los resultados indican que los rendimientos en produccion altamente significativo de las plantas sanas comparadas con las enfermas pueden compensarse por un aumento en la recuperacion de la infeccion, aumento en la incidencia del mosaico en el control y por la siembra de las plantas enfermas en una menor distancia intrahilera que en las plantas sanas.

CONTROL OF SUGARCANE MOSAIC VIRUS BY HEAT THERAPY

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ABSTRACT

The study was carried out to find a suitable method for sugarcane mosaic virus control (SCMV). Hot water (HW), hot Air (HA), aerated steam (AS) and serial hot water (SHW) therapy were used. Only SHW therapy was found to be effective in reducing the incidence of SCMV. At the same time, the reduction in germination percentage was very low as compared to the other therapy methods used.

INTRODUCTION

Heat therapy as a means of control for sugarcane mosaic virus disease has been tested intensively by various researchers in different countries. Vilbrink[9] was the first to use hot water treatment control to such diseases of sugarcane. Since then, numerous other sugarcane diseases including white leaf (Ling and Chuan Young[5], mosaic (Benda[1,2,3 and 4]) and grassy shoot (Zend and Shaikh[10]) have been controlled by heat. Benda[2] concluded that serial hot water therapy controlled the SCMV in some cuttings infected with strain A, B or D. There was also little loss in germination in favorable combinations of treatments. Steib and Cifuentes reported that SCMV and RSD (ratoon stunting disease) could be controlled in one treatment using aerated steam at 56°C for 3 hrs., or 56°C for 3½ hrs., or 57°C for 2 hrs.

In this study, all types of heat therapy were carried out to find out which method is more efficient in eliminating SCMV with the minimum loss in germination.

MATERIALS AND METHODS

Aerated steam:

Canes, 9 months old and with adhering trash of the variety CO419 were used. They were harvested 3 days before treatment and kept in alkathene bags to avoid evaporation and high humidity. Just before treatment, the canes

were cut into 3-eye pieces and were treated in an AS unit at different temperatures (52, 54 and 56°C) for 2 hrs. each. Each treatment was replicated thrice, 20 cuttings per replication. Three–inch long single eye pieces were planted in pots using sandy loam soil. Plants initially planted in 6" x 8" pots were transferred to 10" x 12" pots using the same planting medium. The plants transplanted were fertilized to obtain maximum growth. Treated plants were compared with untreated mosaic checks for appearance of mosaic symptoms. Periodic visual examinations were made over a period of 2 to 3 months.

Hot Air:

Canes from infected plants of Co 419 variety were harvested 3 days before treatment. The whole canes with the adhering trash were treated at 52 and 54°C for 8 hrs. Appropriate control was maintained. The trash was removed and the canes were cut into single eye pieces 3 inches long. All the treated and control setts were planted and kept under the same conditions as mentioned under AS.

Visual observations on the development of the mosaic symptoms were made for a period of 2 to 3 months.

Hot Water:

The infected plants of Co 419 variety were grown for about one year. The stalks were kept for 3 days before treatment under such conditions to avoid evaporation and high humidity. The stalks were cut into 3-eye pieces and treated in a hot water bath at 52°C for 1, 2 and 3 hrs. Suitable controls were maintained. After the treatments, the cuttings were dipped in cold water for 1-2 minutes, dried and kept for 24 hrs. at 27°C. Before planting, the treated and untreated cuttings were cut into single eye pieces and then planted as described before. The observations were made periodically for 2-3 months.

Serial hot water

The experiment was carried out according to the procedures adopted by Benda[2,3,4]. Eight treatments and two untreated control were maintained. The treatments representing different temperature levels and time intervals were as follows:

T_1 – 54.8, 56.6, 57.3 and 57.3°C (7 min. at 24 hr. interval)
T_2 – 54.8, 57.3, 57.3 and 57.3°C (10 min. at 24 hr. interval)
T_3 45.8, 57.3 and 57.3°C (10-10½ min. at 24 hr. interval

} Treatment at 3 days after harvesting

T_4 – 52.0, 57.3 and 57.3°C (20 min. at 2 hrs. interval)
T_5 – Control (untreated)

T_6	as T_1	Treatment at 6 days after harvesting
T_7	as T_2	
T_8	as T_3	
T_9	as T_4	
T_{10}	as T_5	

The hot water bath maintained the required temperature which only varied by 1°C. The cuttings of each treatment were wrapped in a single layer of cheese cloth. There were 48 single eye pieces per treatment representing three replications for each treatment. After treatment, the bundle was dipped in cold water for one or two minutes. The cuttings were allowed to drain briefly and afterwards were covered to reduce evaporation. The treatments were repeated daily at an interval of 22-26 hrs. until the treatment series was completed.

The treated cuttings and controls were planted in a sterilized sandy loam soil and grown in a screened glass house. The seedlings were observed periodically for 2-3 months.

RESULTS

Hot water treatment at 52°C for 1, 2 and 3 hrs. did not have any effect on the elimination of SCMV in treated setts. Germination of setts treated for 1, 2 and 3 hrs. were 55-65%, 40-50%, and 30-60%, respectively. Germination of untreated setts was 80-90%. In the case of hot air treatment at 52 and 54°C, germination were both 15-25% as compared to 75-85% of the control. No single healthy seedlings was obtained from the treatment. Aerated steam also gave no healthy plant. The germination percentage however was proportionally increased with increasing temperature and duration of treatment but not too much difference can be observed in either 1 or 2 hrs, treatments of the same temperature. At 52, 54 and 56°C, the percentage germination were 30-55, 25-50 and 5-20. respectively, The control had 80-90%.

Serial hot water therapy

The effectiveness of serial hot water therapy on 9 and 5 months old setts was tested. The following results were obtained:

Effect on SCMV in 9-month old sugarcane setts

The data (Table 1) showed that although the germination was reduced to some extent, the treatment had significant effect in the production of SCMV healthy seedlings by eliminating the virus. This demonstrated the effectiveness of serial hot water therapy. It can therefore be recommended as a method of control.

TABLE 1. Efficacy of serial hot water (SHW) treatment on the incidence of SCMV in 9-month old sugarcane setts (treatments were carried out at 24 hr. interval

Treatments	% germination[x]			Transformed values of germinated buds				% of healthy seedlings				Transferred values of healthy seedlings				
	RI	RII	RIII	RI	RII	RIII	Total	Mean	RI	RII	RIII	RI	RII	RIII	Total	Mean
1. 52, 57 and 57.3°C for 20 min.	73.3	80.0	63.3	58.9	63.4	52.7	175.0	58.33	36.7	50.0	53.3	37.3	45.0	46.9	129.2	43.3
2. Control (Untreated)	90.0	83.3	86.7	71.6	65.9	68.6	206.1	68.70	0.83	0.83	0.83	5.2	5.2	5.2	15.6	5.2

SE: 2.57 x Germination out of 30 buds for each replication CD: 8.90

'0' values are replaced by : $\frac{100}{4N} - \frac{100}{4 \times 30} - \frac{100}{120} = 0.83$ as suggested by Cochran:

Means

Treatment	Germination	Healthy
T_1	58.33	43.07
T_2	68.70	5.20

Conclusion: $G = T_2 \; T_1$

$H = T_1 \; T_2$

TABLE 2. Efficacy of serial hot water (SHW) treatment on the incidence of SCMV in 5-months sugarcane setts (treatment were carried out at 24 hr. interval).

Treatment	% germination			Transformed values				% of healthy seedlings			Transformed value			
	RI	RII	RIII	RI	RI	RIII	Total	RI	RII	RIII	RI	RII	RIII	Total
1. 54.8, 56.5, 57.3 and 57.3°C for 7-10 min	73.3	76.7	76.7	58.9	61.1	61.1	181.1	56.7	63.3	60.0	48.9	52.7	50.8	152.4
2. 54.8, 57.3, 57.3 and 57.3°C for 7-10 min	86.7	66.7	86.7	68.6	54.8	68.6	192.0	66.7	56.7	73.3	54.8	48.9	58.9	162.6
3. 57.3, 57.3, 57.3°C for 10-10½ min	60.0	73.3	73.3	50.8	58.9	58.9	168.6	60.0	73.3	63.3	50.8	58.9	52.7	162.4
4. 52, 57.3 and 57.3°C for 20 min	66.7	53.3	53.3	54.8	46.9	46.9	148	56.7	40.0	43.3	48.9	39.2	41.2	129.3
5. Untreated Control	86.7	90.0	76.7	68.6	71.6	61.1	201.3	0.83	0.83	0.83	5.2	5.2	5.2	15.6

Germination out of 30 buds in each replication

'0' value is replaced by $\frac{100}{4N} = \frac{100}{4 \times 30} = \frac{100}{120} = 0.83$ as suggested by Cochran.

Means

	Germination	Healthy
T_1	60.37	50.80
T_2	64.00	54.20
T_3	56.20	54.13
T_4	49.53	48.10
T_5	67.10	5.20
	SE : 2.76	CD : 8.21

Conclusion:

Germination = $\overline{5, \underline{2, 1}\quad \underline{3}, 4}$; Healthy = $\overline{2, 3, \underline{1}, \underline{4}, 5}$

TABLE 3. Effect of different periods of storage when given serial hot water treatments (24 hr. interval) on the germination and on the production of healthy material

Treatment		% germination and healthy seedlings after different storage period*			
		% germination		% healthy seedlings	
		3 days	6 days	3 days	6 days
$T_{1,6}$	54.8, 56.5, 57.3 and 57.3°C for 7-10 min	64.5	62.5	50.0	52.1
$T_{2,7}$	54.8, 57.3, 57.3 and 57.3°C for 7-10 min	68.8	39.6	50.0	29.3
$T_{3,8}$	54.5, 57.5, 57.3 and 57.3°C for 10-10½ min	64.5	54.2	47.9	47.9
$T_{4,9}$	52, 57.3 and 57.3°C for 20 min	43.8	37.5	35.4	37.5
T_5	Untreated control	91.7	72.9	0	0

* *Results obtained from 48 buds from three replications with 16 buds for each treatment.*

Effect on 5-month old canes

The effect of treatment T_1, T_2, T_3 and T_4 with different temperatures and time duration combination was studied. The treatment T_2 gave the highest germination (54.8, 56.5 and 57.3°C for 7-10 min.) next to the untreated control and yielded an equal number of healthy seedlings as T_3 and T_1. The lowest percentage germination and the lowest number of healthy seedlings were obtained from T_4 (Table 2).

Effect of storage on the elimination of SCMV

From the results obtained (Table 3) regarding the effect on germination and the production of healthy material there were no significant differences between the treatment when the material were stored for 3 or 6 days. Of the treated seedlings, the maximum germination percentage (68.8%) was obtained from treatment 2 with a 3-day storage period. The least percentage of germination (29.2%) was obtained with treatment 4 when the material was stored for 6 days. It was clear that 6-day storage reduced the germination percentage considerably. At the same time, it had no beneficial effect on the elimination of SCMV unlike those stored for 3 days. It seems that treatment 2 with 3-days storage is the best in terms of high germination and elimination of the disease.

DISCUSSION

The elimination of *clonally* transmitted viruses by subjecting seed material to different forms of heat has been reported to be effective by several workers (Nyland and Goheen[6], Wilbrink[9]; Wang (1957); Ling and Chaung-young[5]; Benda[1]; Thomson[8] and Zendeand Shaikh[10]). The results of such reports were

mainly based on only one type of heat treatment. The four forms of heat treatment were compared as to their relative efficacy in the present study. Serial hot water therapy was the only type of treatment which helped in the elimination of clonally transmitted virus in sugar cane setts. The use of SHT also gave the highest percentage germination particularly when the canes are treated for seven minutes hot water at 54.8, 57.3 and 57.3°C at an interval of 24 hrs. The setts were stored three days before treatment under conditions preventing evaporation and high humidity.

These results coincide with the findings of Benda [1,2,3,4] that a positive result could be obtained with different combinations of temperature and periods of treatment. The role of heat on the virus is still not fully understood, however, and intensive research is required on the physiological changes in enzymes and proteinous substances before and after heat therapy.

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CONTROL DEL VIRUS DEL MOSAICO DE LA CAÑA DE AZUCAR I. – TERAPIA DE CALOR

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RESUMEN

Se usaron terapias tales como tratamientos con agua caliente, aire caliente, aire de vapor y agua caliente seriada para controlar el virus del mosaico de la caña de azucar. Solamente la terapia con agua caliente seriada fue encontrada ser efectiva para reducir el virus del mosaico de la caña de azucar con poca reducion en el procentaje de germinacion cuando comparada con otros metodos de tratamientos.

SOYBEAN *(GLYCINE MAX* MERR.) AND GREEN GRAM *(VIGNA RADIATUS* (L.) WILCZEK) AS LOCAL HOSTS FOR SUGARCANE MOSAIC VIRUS

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ABSTRACT

Soybean *(Glycine max)* and green gram *(Vigna radiatus)* are local lesion hosts for SCMV. Both were used for different quantitative studies for the first time.

INTRODUCTION

There is no information regarding local lesion hosts for sugarcane mosaic virus except that has been reported by Jean Jongho and O. P. Sebgal (1969) and Dean (1970) who stated that atlas sorghum acted as a local lesion host for SCMV–E. They also reported that *Sorghum bicolor* L. cultivars could be used as a local lesion host for maize dwarf virus.

An attempt was made to find out an easy and sensitive local lesion assay host. The method adopted by Pleu (1977) was followed.

MATERIALS AND METHODS

Seeds of different dicotyledonous plants including cultivated ones were sown in pot containing compost soil and kept from sowing date in a room provided with mercury vapor lamp. The room was 2.50 m. high, 1.47 m. wide and 1.60 m. long with bench 0.90 m. from the base. In addition to the door, a black screen made up of cotton cloth was provided to ensure complete darkness. The room was provided with two 100 W mercury vapor lamp being 40 cm apart and 1 m above pots. The plants were exposed to illumination for 16 hours and 8 hours in darkness. The relative humidity in the room was about 75 percent. Tap water was given daily without addition of artificial nutrition. When the plants reached the stage of inoculation (15-21 days after sowing) they were kept in darkness for 24 hours before inoculation. After inoculation they were kept again in darkness for one day. Fully expan-

ded leaves growing horizontally were selected for inoculation. After 4 — 10 days the leaves were observed for the appearance of symptoms, the lesions if formed on plant species were counted. The lesions were cut and ground in phosphate buffer solutions at the concentration of 0.1 M (ph 7.3) and the extracted juice was inoculated as described before on CO. 18 leaves and on the same host to confirm the results. Control plants of the plant species tested were inoculated and kept under day light and fluorescent tubes. Suitable number of the test plant species was also inoculated with buffer only and kept under the same conditions.

An attempt was made to keep suitable number of plants of the same species under natural condition without providing mercury vapor lamp at any stage of the experiment. The plants then were kept on flat benches under glass house condition to see if it is possible to produce local lesions under natural conditions. The results were recorded.

EXPERIMENTAL RESULTS

Local lesion reaction

(i) Green gram CV. CO. 1, CO.2 and CO.3 *(Vigna radiatus)*

On the inoculated primary leaves, oval irregular purple local lesions which enlarge rapidly to form larger local lesions could be observed 2 to 3 days after exposure to mercury vapor lamp illumination the leaves dried up. Generally 2 to 3 lesions were observed on each leaf (Plate 1). Green gram was found to be suitable assay host during summer time when the temperature was above 28°C.

PLATE 1

An attempt was made to find out whether exposure to mercury vapor lamp illumination was essential for the development of local lesion on green gram by leaving the plants under natural reflected light. It was observed that within 24 hr. of inoculation purple round local lesions were produced on the cotyledonary leaves. Within 2 to 3 days the lesions coalesced to cover the entire leaf and then shedding of the leaves started, after another 2 to 3 days the whole leaves dried up. When the inoculum was prepared from tissues with local lesions and inoculated back to healthy seedlings, it produced the same local lesions as the original inoculum did. When it

was inoculated on *Sorghum bicolor* L., CV. CO.18, it produced mosaic symptoms within 15 days of inoculation. When green gram plants were left to produce true leaves and the cotyledonary leaves were inoculated, large number of small round purple lesions were observed on the true leaves within 24 to 48 hr after inoculation. The number of lesions ranged from 20 to 40 and only 2-3 lesions were observed on the inoculated lesions.

(ii) Soybean — *Glycine Max.* Merr. CV. UGM20

The host reacted with small well defined yellow lesions which turned necrotic with reddish margin local lesions formed were numerous and they developed within 3 days after the exposure to mercury vapor lamp illumination. The number of lesions per leaf ranged from 25 to 60 lesions. No lesions appeared when soybean leaves were inoculated with the juice extracted from healthy sugarcane plants. The lesions were produced only on the true leaves, cotyledons failed to show any symptoms (Plate 2).

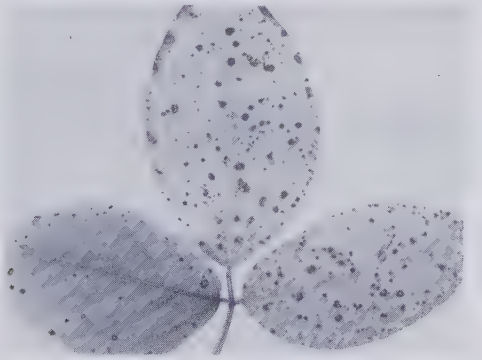

PLATE 2

The type of lesions produced on both hosts was defined as translocated necrotic local lesions (TNL).

In order to make use of local lesion assay in the study of other aspects, it was necessary to find out the proportional increase or decrease in the number of lesions with increasing dilution. The experiment was carried out by extracting the juice from SCMV infected leaves of sorghum. The juice was squeezed through cotton pad. Then was purified by subjecting juice to one cycle of low and high centrifugation for a period of 1 and 2 hr, respectively. One ml. of the aliquot was taken and the dilution with 0.1 M potassium phosphate buffer at ph 7.3 were made as follows: 1:10, 1:100, 1:500; 1:1000, 1:10.000 and 1:100.000. Soybean and green gram leaves were dusted with 600 mesh carborundum and inoculated with different dilutions. The inoculated leaves were washed after 10 — 15 min of inoculation and then were kept in dark for 24 hr. Within 2 days of inoculation for green gram and 3 days for soybean the number of lesions was counted as shown in Tables 1 and 2.

The following hosts did not show any lesions either in the presence or absence

of mercury vapor lamp illumination.

Family	Name of the host
Chenopodiaceae	*Chenopodium album* L
	C. amaranticolor Cost et Reyn.
Cruciferaceae	*Raphanus sativas* L.
Leguminaceae	*Lablab niger* medikus
	Phaseolus verlgris L
	Vicia fabee L.
	Vigna sinensis L
Solanaceae	*Capsicum annum* L.
	Datura stramonium L.
	Lycopersicon lycopersicum Mill
	Nicotiana glutinosa L.
	N. tabacum L.
Family (?)	*Tridex procumbens* L.
	Zinnia elegans tacop.

TABLE 1. Number of local lesions on soybean induced by juice inoculation from mosaic infected and healthy sugarcane

Mean number of local lesions on soybean	
Infected juice	Healthy juice
47.7	0

* *mean of six replications*

Detection of latent infection:

Detection of the virus during pre- and post — harvest stages is very important to eliminate the use of infected canes for planting purposes and this may check the disease to great extent. The infected inter nodal tissue (10 g) was crushed using mortar and pestle and the extracted juice was inoculated on soybean leaves which were previously kept in darkness for 24 hr. After another 24 hr. of inoculation, the inoculated seedlings were exposed to mercury vapor lamp illumination. Suitable control soybean seedlings were inoculated with juice extracted from healthy canes after 3 to 4 days, the translocated necrotic local lesions were counted on the three leaves of the seedlings inoculated with juice from suspected tissues (Table 3), indicating the efficacy of local lesion assay method for detection of latent infection.

"Distribution of SCMV in different parts of the sugarcane plants"

The distribution of SCMV in roots, and different internodal portions of the cane was studied in plant as well as in ratoon crops of the variety CO.419. Ten g of

infected root, internode and rind of each internode were ground separately using mortar and pestle. The juice was extracted in 0.1 M potassium phosphate buffer at pH 7.3 and the extracted juice was partially filtered through cotton pad. Soybean seedlings previously kept in dark room for 24 hr. were inoculated with inocula prepared from different tissues. The inoculated leaves were washed and the plants were kept again in darkness for another 24 hr. Then they were exposed to mercury vapor lamp illumination.

TABLE 2. Distribution and titre of SCMV in infected sugarcane plant

Serial number from the base of the cane	Plant crop				Ratoon crop			
	Mean number of lesions X				Mean number of lesions X			
	Rind	Internode	Rind + internode	% increase of internode over rind	Rind	Internode	Rind + internode	% increase of internode over rind
Root XX	4.17	—	—	—	6.00	—	—	—
1 st	3.33	14.17	17.67	325.50	4.00	16.67	20.67	316.75
2 nd	7.50	15.00	22.50	100.00	4.33	22.83	27.17	427.25
3 rd	13.50	27.00	40.50	100.00	18.33	33.17	51.50	80.96
4 th	14.50	27.33	61.83	88.48	10.50	34.17	44.67	225.42
5 th	12.67	51.53	64.50	314.60	14.17	57.00	71.17	302.25
6 th	12.50	36.83	49.33	194.64	14.33	47.17	61.50	229.15
7 th	14.33	24.50	38.83	70.96	16.33	34.67	51.00	112.30
8 th	10.83	22.50	33.33	107.75	12.83	24.50	37.33	90.95
9 th	9.83	18.83	28.67	91.50	7.50	19.67	27.17	126.26
10 th	7.33	19.00	26.33	159.20	7.00	19.33	26.33	176.14
11 th	10.00	19.33	29.33	93.30	6.83	20.33	27.17	196.66
	S.E.	O.						
S.E.	0.78	0.81	1.13		1.08	0.60	1.16	
C.D.	2.21	2.28	3.20		3.05	1.70	3.28	

X *Mean of six replications*

XX *Root is taken as one portion as a whole*

TABLE 3. Concentration and distribution of SCMV in the buds of variety Co.419

Serial number of buds from the base	Plant crop			Ratoon crop		
	Weight of budding	No. of lesions per actual weight	No. of lesions per 10 g	Weight of budding	No. of lesions per actual weight	No. of lesions per 10 g
1 st	0.75	1	14.2	0.64	3	46.8
2 nd	0.65	1	15.4	0.73	4	54.7
3 rd	0.57	2	35.0	0.70	5	71.4
4 th	0.57	4	70.0	0.82	18	219.5
5 th	0.57	10	175.4	0.61	28	459.8
6 th	0.62	33	532.2	0.61	38	622.9
7 th	0.52	39	741.3	0.70	63	900.0
8 th	0.40	27	675.0	0.60	47	783.3
9 th	0.35	25	714.2	0.70	55	783.3
10 th	0.30	21	700.0	0.55	46	836.3
11 th	0.22	6	272.7	0.46	34	739.1
12 th	0.20	3	150.0	0.65	41	630.7
13 th	0.20	3	150.0	0.72	43	597.5

The number of translocated necrotic local lesions (TNL) found on leaves inoculated with different inocula are furnished in Tables 4 and 5.

A) *Plant crop:* The virus concentration present in different tissues as determined by the number of lesions showed significant variation (Table 4). The roots contained the lowest virus concentration. The concentration of the virus reached in the internodal tissues progressively increased when assayed from the root portion to the top. The virus titre reached the maximum in the fifth

TABLE 4. Effect of sucrose on the infectivity of SCMV as expressed by number of lesions on the assay hosts

Treatment	x Mean number of lesions on soybean	x Mean number of lesions on green gram
Sucrose 5%	23.67	19.83
Sucrose 10%	62.50	32.83
Sucrose 15%	72.67	39.5
Sucrose 20%	119.16	41.67
Phosphate buffer	46.00	26.67

x Mean of six replications

SE	*1.63*	*0.86*
XD	*4.82*	*2.54*

Conclusion: 4, 3, 2, 5, 4 4, 3, 2, 5, 1

TABLE 5. Correlation between sucrose content of the variety and its susceptibility to SCMV as expressed by number of lesions on soybean

Variety	Sucrose %	x Mean number of lesions		
		Leaf tissue (10 g)	Internode tissue (10 g)	Bud tissue (10 g)
Co. 419	16.19	103.33	57.33	112.00
Co. 740	17.23	106.00	62.00	281.33
Co. 658	16.16	72.67	56.00	110.67
C.P. 29/320 x US. 49/7	18.69 3.17	0.00	0.00	18.67

x Mean of three replications, and each replication six leaves

S.E.	*2.70*	*0.87*	*8.37*
C.D.	*9.35*	*3.00*	*28.97*

Conclusion 2, 1, 3, 4 2, 1, 3, 4 2, 1, 3, 4

internode and the concentration decreased in the internodes above. However, 11th internode had higher virus concentration than the first two internodes. The internodal tissue contained significantly greater virus content than the rind, the increase in virus content ranged from 70.96 to 325.50 per cent.

B) *Ratoon crop:* In ratoon crop also, significant differences in the distribution of SCMV in different tissues were observed. A similar trend in the virus titre on plant crop was observed in ratoon crop. The ratoon crop had higher virus concentration when compared to plant crop.

Concentration and distribution of SCMV in the buds of sugarcane plant:

Every bud was removed separately along with a small portion of the tissue present around the buds using a sharp grafting knife. The bud was cut into very small bits with a sharp razor blade and was ground using mortar and pestle which were kept in ice tray throughout the procedure of extraction and inoculation. Potassium phosphate buffer (0.1 M) at pH 7.3 was used as the extraction medium. The extracted juice was inoculated on soybean leaves which were previously dusted with carborandum. The soybean seedlings were kept in darkness for 24 hrs. before and after inoculation. The inoculated leaves were washed with water 10 to 15 min after inoculation. After 2 to 3 days of exposure to mercury vapor lamp illumination, the lesions were counted and from the number counted, the lesions produced by 10 g of bud tissues were calculated. Suitable number of soybean seedlings were inoculated with potassium phosphate buffer alone to serve as control.

The data obtained presented in Table 3, indicated that the buds contained higher virus titre than other tissues. The virus titre increased considerably in ratoon crops; the seventh bud record the highest concentration in both plant and ratoon crop; SCMV was detected even in the upper most buds viz; 10, 11, 12 and 13. The titre of the virus in those buds was high in ratoon and low in the plant crops. It can be concluded from the present study that the SCMV was distributed in all parts of the sugarcane plant showing the difference in the concentration of the virus indicating the differences in the ability of the tissue to support virus multiplication.

Effect of sucrose on the infectivity of SCMV on the assay host plants:

To find out the relationship between sucrose content of sugarcane cultivars which also differ in their resistance to SCMV and the incidence of SCMV, the following experiment was carried out.

Solutions with different sucrose concentrations viz, 5, 10, 15 and 20 per cent were prepared and used as extraction media instead of potassium phosphate buffer which were used as a control to the present study. Five g of infected leaf tissue was taken as a uniform weight for each treatment. The leaf tissues were ground and the juice was extracted in the above mentioned sucrose solutions. In one treatment the extraction was done with the help of 0.1 M potassium phosphate buffer at pH 7.3 which acted as control. Immediately, the extracted juice. was inoculated on cotyledonary leaves of soybean and green gram seedlings which were previously kept in darkness for 24 hrs.The assay host leaves were dusted with very fine car-

borandum powder before inoculaton. The seedlings were kept again in darkness for 24 hrs. after which the seedlings were exposed to mercury vapor lamp illumination. Within 1 to 3 days, the TNL were clear enough to be counted on the true leaves of both the hosts.

From the data obtained in (Table 4) it is clear that the maximum number of lesions was recorded when 20 per cent sucrose solution was used as extraction medium followed by 15, 10 per cent and potassium phosphate buffer. The lowest number of lesions was observed when 5 per cent sucrose solution was used for extraction. This indicated that there is a positive relationship between the number of lesions and the concentration of sucrose solution.

When different varieties of sugarcane containing different percentage of sucrose were tested on the local lesion assay host, the number of lesions produced was correlated positively with sucrose percentage of each variety. It is clear from the data in (Table 5) that the hybrid (C.P. 29/320 X U.S. 4917) which is known to be resistant gave an average of 18.67 lesions when bud tissues were used and no lesion when leaf and internodal tissues were used for inoculation whereas Co. 740 recorded the highest number of lesions followed by Co. 419 and CO.658.

This clearly shows that sucrose acts as preservative for SCMV and also indicates that a high sucrose content is associated with susceptibility of the variety to SCMV. The detection of some lesions in the case of resistant hybrid may be due to the presence of high sucrose content (18-69 per cent) in the blood of the parent C.P. 29/320 which when crossed very low sucrose content parent (3:17 per cent) viz; U.S. 4917 produced the resistant hybrid. These results indicate that there is no absolute immunity in plants.

DISCUSSION

The significant findings of Holmes (1929) per se contributed to a great extent for the development of quantitative assay of plant viruses. Since then numerous investigations have been carried out to find out the possibility of obtaining a host plant which would react with local lesions which reflect the possible variation in the virus titre. Such studies have been instrumental in gathering knowledge on various aspects of plant viruses whose infection of plants is influenced by various factors, the effect of which could determine the bioassay methods based on the infectivity of the virus preparations. The sap transmissibility of viruses to a given plant species is affected by several genotypic and environmental factors in which the host plant grown. Several studies have indicated that it is quite possible by providing suitable optimal conditions, the sap transmissibility of a plant virus can be effected.

An earlier study (1977) gave an impetus to the line of investigation aiming at the development of suitable assay host.

In the present study, attempts were made to explore the possibility of finding out an assay host for the sugarcane mosaic virus. Translocated necrotic lesions (TNL) were obtained on soybean and green gram on the second trifoliate leaves

as seen in Plates 1 and 2. It is worth mentioning that green gram developed TNL within 3 – 4 days after inoculation without exposing the seedlings to mercury vapor lamp illumination.

The translocated necrotic lesions has similarity with the observation of Yohu – 1969) who got TNL on *Chenopadium almum* and *C. amarnticolor* when sap inoculated with TNL or the Japanese strain of Turnip yellow mosaic virus on the upper young leaves after 10-30 days of inoculation. Though the formation of local lesions by viruses infecting graminaceous or monocotyledon host is known in the case of barley stripe mosaic virus (BSMV) which caused local lesions on different barley varieties (McKinney, 1951).

The present study records for the first time the formation of local lesion on dicotyledonous host plant species by a virus infecting a graminacrous host. This finding has opened up a new vista of possibilities of studying the effect of various chemicals and environment on the virus multiplication as well as the process of virus infection.

It facilitated study of the titre and distribution of the virus in different parts of the cane. In addition to that, the effect of different concentrations of sucrose on the infectivity of sugarcane mosaic. The results obtained showed a positive relationship between the number of lesions and the concentration of sucrose solution which explain the significant possibility that sucrose acts as a preservative for the virus and enhances the multiplication and productivity of SCMV inside the cane plants. The present study revealed that the high sucrose content varieties are highly susceptible to SCMV and *Vice veria.* The present investigations explains why the breeding for resistance failed up till now to offer a commercial high sucrose content variety and this in turn proved that selection of healthy material or establishing sugarcane nurseries surrounded by barrier crop like sunflower are the most effective solutions for eliminating SCMV and accordingly maintaining of the standard varieties for a long period.

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SOYA *(GLYCINE MAX* MERR.) Y GRAMO VERDE *(VIGNA RADIATUS* (L.) WILCZEK, COMO HUESPEDES DE LESION LOCAL DEL VIRUS MOSAICO DE LA CAÑA DE AZUCAR

S. H. Farrag, T. K. Kandasamy y P. Narayanasamy

RESUMEN

Se descubrio que la soya y gramo verde son muy sensitivos cuando se usan como huesped de la lesion local del virus mosaico de la caña de azucar (SCMV) y se usaron para diferentes estudios cuantitativos por primera vez.

FUNGUS PATHOLOGY IN SUGARCANE CARYOPSES

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ABSTRACT

In the evaluation of occurrence of fungi in sugarcane caryopses, 73% of examined caryopses were infected. The genera *Cladosporium*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Pestalotia* and *Phoma* were among the most important.

The pathogenicity of each fungus species found in the caryopses was tested. The species *Fusarium moniliforme*, *Pestalotia guepini*, *Cladosporium* (probably *herbarum*) and *Phoma sp*, were considered pathogenic and seed borne on sugarcane caryopses.

INTRODUCTION

The determination of fungi that infect and contaminate seeds, causing germination failures and death of seedlings has been carried out in cereal and other sugar. In sugarcane, the production of fuzz is restricted to the variety improvement program and the normal propagation of sugarcane in the fields is by vegetative means.

Usually, the ideal relative humidity and temperature for flowering of sugarcane are also ideal for the development of fungi which attack caryopses causing a poor caryopses formation, germination and consequently, seedling death.

MATERIALS AND METHODS

Sugarcane caryopses, from the COPERSUGAR Experiment Station in Camamu-BA., received a pre-cleaning treatment (Silva[11]), by ventilation in seed blowers to eliminate the husks (palea and lemma) and rotten spicules. One hundred fertile caryopses were germinated in petri dishes with 25 ml of water-agar and incubated for 6 to 8 days at 28°C, under a photoperiod of 12 hours.

For determination of fungi occurring in the husks, and in the caryopses, the

caryopses were separated from the husks by pressing a stylet against the basal end of the fertile spicules till the caryopses were removed. The dehusked caryopses were soaked for 3 minutes in a solution 1:3 V/V of sodium hypochlorite (with 5% active chlorine), before placing it in petri dishes with water-agar. The husks were placed into separate petri dishes with water-agar for fungi determination.

The fungi potentially known as pathogenic and most frequent were identified to species and others only to genus level.

The fungi isolated from the caryopses were cultured in cane leaf agar (Wismer and Koike[17]) and their pathogenicity were tested. Seedlings used in pathogenicity tests were obtained from seeds treated with formaldehyde (40% formaldehyde, diluted with water 1:20 V/V) for 20 minutes and germinated in petri dishes with water-agar. After germination, only fungus-free seedlings were

TABLE 1. Genera of fungi and number of colonies found in husks and caryopses of 400 sugarcane seeds

Genera of Fungi	Caryopses		Husks of Seeds	
	Number of colonies	%	Number of colonies	%
Alternaria spp	4	1.00	8	2.00
Cladosporium spp	36	9.00	47	11.75
Curvularia spp	56	14.00	71	17.75
Fusarium spp	46	11.50	43	10.75
Helminthosporium spp	63	15.75	83	20.75
Monilia spp	15	3.75	36	9.00
Nigrospora spp	0	0.00	4	1.00
Pestalotia spp	21	5.25	4	1.00
Phoma spp	41	10.25	64	16.00
Pithomyces spp	4	1.00	27	6.75
Rhizopus spp	0	0.00	20	5.00
Tricoderma spp	0	0.00	15	3.75
Undetermined	6	1.50	8	2.00
Total Infected	292	73.00	430	107.50

transplanted (Silva[11]) into 2 substrata, one containing sterilized soil and the other in petri dishes with water-agar. Fifty seedlings were inoculated separately by each fungus. The inoculum for each fungus was prepared by blending the 20-day old culture in an electric blender with little water, and then poured over seedlings to be inoculated.

The inoculated seedlings were kept in a humid chamber for 48 hours and after 10 days the seedlings were examined for disease symptoms and reisolation of the fungus from the diseased tissues were carried out.

RESULTS

The fungi found in caryopses and husks of 400 fertile seeds were identified to the genus level and their relative abundance as indicated by the colonies formed were determined. The results are shown in Table 1.

The results of pathogenicity test is shown in Table 2. The fungi used caused leaf spots, color and root rots and death of seedlings.

DISCUSSION AND CONCLUSIONS

Determination of fungi present in caryopses and husks of sugarcane seeds

Of the 400 caryopses examined, 73% were infected with fungi. The infection in seed husks was higher because of the occurrence of 2 different fungi on the same husk. Of the 400 husks tested, 430 different fungal colonies were produced. The results in Table 1 show that 13 genera of fungi are present in husks of seeds. In caryopses, the genera *Cladosporium, Curvularia, Fusarium, Helminthosporium, Pestalotia* and *Phoma* showed percentages of infection varying from 5.25% and 15.75%. In general, the same pathogenic fungi found in caryopses also occur to a high frequency in seed husks.

Pathogenicity test of fungi found in caryopses

Among the fungi found in caryopses and in husks of sugarcane seeds, those which occurred in high frequency were selected, as well as those described as pathogenic to other species of cultivated plants.

In general, the tested fungi produced higher infection in inoculated seedlings grown in water-agar than those in soil (Table 2). This could be due to the fact that the water-agar promotes more contact between the inoculum and the seedlings, besides promoting a rapid multiplication of fungi. As such, all seedlings are in contact with a great number of inoculum. The low infection on seedlings grown in soil maybe attributed to the fungistic effect of soil microorganism, and also the periodic irrigations may have washed away a great number of inoculum thus reducing infection.

TABLE 2. Pathogenicity test of some fungi isolated to sugarcane seedlings growing in two substrata in sterile soil and plated water agar

Fungi Inoculated	Isolate number	Seedling Symptoms									
		Leaf spot		Collar rot		Root rot		Dead		Total with symptom	
		Agar	Soil	Agar	Soil	Agar	Soil	Agar	Soil	Agar	Soil
Cladosporium spp	1	0	1	1	1	0	0	3	0	4	2
	2	0	0	4	1	1	0	3	0	8	1
	3	0	2	2	1	2	0	4	2	8	5
Cuvularia spp	1	6	1	10	7	4	3	6	5	26	16
	2	5	3	11	9	2	4	5	2	23	18
	3	7	3	6	4	4	3	3	1	20	11
C. lunata	1	12	7	14	10	2	1	5	4	33	22
	2	9	3	6	5	1	2	4	3	20	13
	3	5	1	7	6	3	1	4	1	19	9
Fusarium spp	1	0	1	6	6	1	2	4	2	11	11
	2	0	0	7	8	2	3	4	1	13	12
	3	0	1	6	5	1	1	4	3	10	11
F. moniliforme	1	0	1	8	7	6	4	7	4	21	16
	2	2	0	10	12	8	7	6	2	26	21

TABLE 2. contd.

Helminthosporium spp	1	12	6	5	3	3	2	8	3	28	14
	2	7	4	3	1	2	1	3	2	15	8
	3	10	6	8	1	2	3	2	1	22	11
H. rostratum	1	14	5	7	2	2	1	5	2	28	10
	2	7	4	2	0	3	1	6	4	18	9
H. sacchari	1	20	10	8	6	5	3	4	3	37	22
	2	11	10	6	5	2	1	1	3	20	19
	3	16	7	4	3	1	1	5	4	26	15
Pastalotia quepini	1	4	1	2	1	1	0	2	3	9	5
	2	5	2	1	2	1	1	1	0	8	5
Phoma spp	1	2	0	1	0	0	1	3	3	6	4
	2	4	0	0	0	0	0	2	1	6	1
	3	1	0	0	0	0	0	2	1	3	1
Check		1	0	0	0	0	0	0	0	1	0

Note: Fifty seedlings were inoculated for each fungus isolate.
The leaf spot and collar rot developed until causing seedling death, and root rot caused immediate drying of aerial part.

Results of pathogenicity, *Helminthosporium sacchari, Helminthosporium rostratum* and *Curvularia lunata* correspond to the data obtained by Loveless and Smith[6] and Byther and Steiner[3]. These authors considered these fungi as the main cause of seedling deaths in seed flats. The need to control these pathogens for a better caryopses germination has been acknowledged. The disease symptoms observed in our test were very similar to those described by Byther and Steiner[3].

No literature was found on *Fusarium moniliforme* causing the seedling death and its transmission by caryopses of sugarcane. This report is apparently the first. *Pestalotia guepini, Cladosporium* sp. and *Phoma* sp. had low pathogenicity, but they can increase the total mortality when acting together with other pathogens.

Besides the species identified, other unidentified species of the genera *Helminthosporium, Curvularia, Fusarium* and *Cladosporium* occur in sugarcane caryopses.

The fungus survey in caryopses indicated that a great number of seedling death after germination and transplant was due to the pathogenic species carried on the seeds.

The species *H. rostratum* was found highly pathogenic to caryopses and seedlings and these results were similar to the data obtained by Byther and Steiner[3,4] in Hawaii. It can cause seedling blight and foliar spots in various species of gramineous plants (Whiteheard and Calvet[15], Smith[12]). Therefore these plants can be the source of inoculum to contamination of sugarcane seeds. Similar results were found in Hawaii with *Curvularia lunata* which was described as one of the main pathogens of sugarcane seedlings (Byther and Steiner[3]). It was also mentioned as the causal agent of seedlings blight in other gramineous plants among which are rice crops and sorghum (Agulero *et al*[1])

The species *Fusarium moniliforme,* shown as being highly pathogenic in caryopses of sugarcane, is known as the causal agent of "Pokkah boeng" and stalk rot in older cane plants (Martin *et al*[7]). "Pokkah boeng" occurs under conditions of high moisture. *Fusarium moniliforme* is a common seed pathogen of other gramineous plants such as rice, wheat, sorghum and corn; (Kucharek and Kommedahl[5], Widhyasekaran *et al*[14], Ram Nath *et al*[9], Winter *et al*[16] Neegard[10] and Aguilar[2]). *Fusarium* is frequently associated with the embryo, interfering with germination and seedling development of grasses. In *Sorghum vulgare,* Mathur and Nath[8] reported *Pestalotia guepini* pathogenic on seeds and seedlings. The fungus *P. guepini, Cladosporium* sp. (possibly *C. herbarum*) and *Phoma* sp., which were found in sugarcane seeds have a low pathogenicity, but they are cosmopolitan and attack leaves and seedlings of sorghum and corn (U.S.D.A.[13]). No report on these genera in sugarcane has been made.

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HONGO PATOLOGÍA EN EL CARIOPSES DE LA CAÑA DE AZUCAR

Alvaro Sanguino y Hasime Tokeshi

RESUMEN

Este trabajo presenta data sobre la evaluacion de la ocurrencia del hongo en el cariópsis de la caña de azucar, donde setenta y tres por ciento del cariopsis examinados fueron infectados.

Los generos del hongo *Cladosporium*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Pestalotia y Phoma* estan entre los mas importantes.

La patogenicidad de cada una de las especies del hongo que se encontro en el cariopsis fue probado. Las siguientes especies *Fusarium moniliforme*, *Pestalotia guepini*, *Cladosporium* (porbablemente *herbarum)* y *Phoma* sp. fueron consideradas patogénicas y portado por la semilla con el cariopsis de la caña de azucar.

CONTROL OF FUNGI FROM SUGARCANE CARYOPSES

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ABSTRACT

The control of fungi from sugarcane caryopses was done in an attempt to identify fungicides with and without long residual effect. Of the four compounds used in different concentrations and time of exposure, thiram 2g/l and formalin 40%, diluted 1:20 v/v were the most effective when used for 20 minutes. Thiram was also recommended in case a residual effect is needed and formalin when no residual effect is desired.

INTRODUCTION

Many studies on the seed pathology of crops have been conducted. In sugarcane, the control of fungi which cause seedling diseases is restricted to soil treatment and application of fungicide with long residual effect such as organic mercury compounds.

Based on studies by Loveless *et al*[2], Singh *et al*[5], Byther *et al*[1] and Sanguino[3] the seed born fungi are responsible for low germination, short storage period, rapid decrease of seed vigor and seedlings death during the early growing period.

As in all other field crops, the system to control sugarcane fungi depends on the species of pathogens present and the form of application of caryopses after the treatment.

The control measure adopted in this research was an attempt to solve three problems: a) control of pathogenic fungi without seedling phytotoxicity using fungicides with large residual effect; b) control of pathogenic fungi with fungicides with low residual effect permitting posterior caryopses inoculation with specific sugarcane pathogens such as *Ustilago scitaminea, Colletrotrichum falcatum* and *Fusarium moniliforme;* c) determination of fungi that were not controlled even by the more effective fungicides.

MATERIALS AND METHODS

Caryopses utilized in this research were a mixture from several crosses and representative of seed used in current breeding program. The caryopses were submitted to a pre-cleaning treatment by Silva's[4] method. To select fertile caryopses, viable seeds were pressed with a needle.

The fungicides used were benomyl (Benlate 50% WP), mancozeb (Dithane M-45 WP), thiram (Arasan 50 WP) and formalin 40% (formaldehyde solution 37%). The first three were applied at a rate of 1 and 2 grams per liter of water. Formalin was used in three dilutions, 1:10, 1:20 and 1:50 v/v with water. The soaking time varied from 0 to 60 minutes.

To treat caryopses with fungicide, they were placed in small nylon bags and soaked in fungicide suspensions during the desired period of time. The control received water treatment. After soaking, the caryopses were rapidly dried over sterile porous ceramic plate to absorb the excess fungicide.

The treated caryopses were sown in 1.8% plated sterile water sugar, incubated for 6 to 7 days at 28°C with alternate 12 hours of light and dark period. Each plate had 50 fertile caryopses, 4 or 5 replications in a completely randomized distribution. The results were evaluated by percentage of germination. Seedlings with well developed radicle and caulicle were considered as germinated caryopses.

To determine the pathogenic fungi that escaped the fungicide treatment, experiment was set in the same conditions described. Each plot had 50 caryopses, 5 replications and in a completely randomized distribution.

RESULTS AND DISCUSSION

Fungicide with high residual effect

The fungicide benomyl, thiram and mancozeb, in general, increased germination. At the rate of 1 and 2 grams per liter only thiram gave a significantly higher germination percentage than that of the control (Table 1). Other treatments were similar to the control. Better germination was probably due to thiram broad spectrum.

Benomyl and mancozeb did not seem to be effective against fungi belonging to the genera *Helminthosporium* and *Curvularia* and they were not phytotoxic to sugarcane seedlings. The data obtained from benomyl treated caryopses were similar to that found by Vir Dharam *et al*[6] and Byther and Steiner. The result from mancozeb presented by Vir Dharam[6] with barley, rice and oat, was excellent. This was not the case with sugarcane caryopses possibly because of fungus resistance to mancozeb.

TABLE 1. Germination of sugarcane caryopses treated for 20 minutes with fungicides with long residual effect

Fungicides	Concentration gram/1	Germination average %
Benomyl	2.0	32.40 ab
	1.0	31.05 ab
Mancozeb	2.0	29.62 ab
	1.0	28.93 ab
Thiram	2,0	33.91 a
	1,0	32.84 a
Control	0.0	26.84 b
	C.V. % = 13.06	

**Averages obtained form 250 caryopses and means with the same letters are statistically similar by Duncan test at 5%.*

Fungicide with low residual effect

Results presented in Table 2, shows that formalin diluted 1:10 reduced germination. The control was statistically superior to all treatments at this dilution. This result indicated that formalin 1:10 caused good fungi control but also caused injuries to seed germination and these injuries increased with time of exposure.

Formalin, diluted 1:20, showed no significant beneficial effects in germination although formalin 1.20 for 20 minutes of exposure resulted to an excellent control of seed borne fungi and some increase in germination. Treatment with formalin diluted 1:50, for 10 to 20 minutes exposure increased germination and was statistically superior to all treatments. All treatments with formalin 1:50 were a fungistatic because later, the fungi on water-agar medium grew with different intensities depending on the time of exposure.

Fungi not controlled by the more effective fungicides

The pathogenic fungi prevalent on the control were *Curvularia* spp, *Helminthosporium* spp, *Phoma* spp, *Cladosporium* spp, *Fusarium* spp. and *Pestalotia* spp. The total number of fungal colonies in the control was considered 100% for comparisons (Table 3). The fungi genera *Curvularia, Helminthosporium, Cladosporium* and *Phoma* were the most prevalent and were controlled

TABLE 2. Germination of sugarcane caryopses treated with different dilutions of formalin 40%

Formalin 40% dilution	Minutes of treatment	Germination average %*
1:10	0	33.35 a
	15	21.34 b
	30	15.71 bc
	45	10.49 c
	C.V. % 23.05	
1:20	20	35.48 a
	40	29.15 a
	0	27.57 a
	C.V. % 17.82	
1:50	20	41.54 a
	10	38.62 ab
	60	32.46 bc
	50	32.35 bc
	40	29.62 c
	0	25.86 c
	C.V. % 13.21	

** Averages with the same letters are statistically similar by Duncan test at 5%. Means estimated from 200 caryopses for formalin 1:10 and 1:50; 2:50 caryopses for formalin 1:20.*

only by thiram 2g/l and formalin diluted 1:20 within 20 minutes of exposure. *Fusarium* was easily controlled by almost all fungicide treatments except by formalin treatment 1:50. *Pestalotia* was controlled efficiently by all the fungicide tested.

In the treatment with mancozeb, 40% of the treated caryopsis were free of fungi of the infected caryopses, of the fungi present belonged to the genera *Curvularia* and *Helminthosporium.* These data did not agree with those obtained by Vir Dharam *et al*[6], indicating that *Helminthosporium* species in sugarcane caryopses are more resistant to mancozeb.

TABLE 3. Determination of fungi that escaped from sugarcane caryopses treatment

Genera of fungi	Number of fungal colonies found in caryopses treated with fungicide					
	Formalin 1:50	Formalin 1:20	Thiram 2g/l	Benomyl 2g/l	Mancozeb 2g/l	Control*
Cladosporium spp	8	0	2	1	5	8
Curvularia spp	12	0	1	7	8	16
Fusarium spp	6	2	1	2	5	7
Helminthosporium spp	10	1	2	6	5	14
Pestaloria sp	0	0	0	0	0	2
Phoma sp	11	2	1	1	4	13
Total	47	5	7	17	24	60
%	78.33	8.33	10.16	20.83	40.0	100

* *Each treatment had 250 caryopses. The control was considered of 100% infection. Period of treatment was 20 minutes.*

CONCLUSIONS

None of the fungicide treatments used gave control of sugarcane caryopses pathogen. The elimination of some genera and species of fungi was sufficient to improve caryopses germination.

In sugarcane and in other gramineous crops, it is almost impossible to eliminate fungi from caryopses embryo. This means that the control of fungi should start before the blooming period to avoid infection during the caryopses formation.

Of the fungicides used, the most satisfactory were thiram 2 g/l and formalin 1:20 for 20 minutes exposure. These treatments controlled almost all the fungi reducing the percentage of infection to 10:16% and 8.33%, respectively without phytotoxic effect on the seedlings. Thiram, because of its residual effect and other specific characteristics was considered more beneficial. Formalin diluted 1:20 is recommended in case no residual effect is desired in caryopses.

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EL CONTROL DE LOS HONGOS EN CARIOPSES DE LA CAÑA DE AZUCAR

Alvaro Sanguino y Hasime Tokeshi

RESUMEN

Se probó del control de los hongos en el cariopses de la caña de azucar con el propósito de conseguir un producto con y sin efecto residual prolongado. Entre los cuatro compuestos que se usaron en diferentes concentraciones y tiempo de exposicion, thiram a 2 g/1 y formalin (40%) en una dilucion de 1:20 v/v fueron los mas efectivos cuando se usaron por 20 minutos respectivamente. Thiram tambien se recomendo para el tratamiento cuando es necesario el efecto residual y formalina se recomendó para cuando no se desea el efecto residual.

SAPROPHYTIC BEHAVIOUR OF *PLEOCYTA SACCHARI* CAUSE OF RIND DISEASE OF SUGARCANE

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ABSTRACT

Pleocyta sacchari grows and sporulates well on a number of agar media. Variation in linear growth rate, growth density and sporulation indicates exacting nutritional requirements. The fungus forms sclerotia on potato dextrose, oat meal and cane juice agar. Growth on mineral media is considerably enhanced by the addition of 0.1% yeast extract.

The competitive saprophytic ability (ČSA) as estimated by both agar plate and Cambridge method suggests that *P. sacchari* is a moderate competitive saprophyte.

P. sacchari actively decomposes cellulose of filter paper and sugar cane bagasse. This is significant as far as the industrial qualities of stored bagasse are concerned. The fungus does not produce pectolytic culture filtrate. It is susceptible to antibiotics produced by soil microorganisms.

Sclerotia produced on agar media and cellulosic substitutes are formed of a number of *pycnidia.* The fungus can survive in pure cultures grown on sterile supplemented sorghum, grains and cellulosic substrates for more than 16 months. It is suggested that the fungus survives in nature growing on plant tissue remains or in the form of *sclerotia* and *chlamydespores.*

INTRODUCTION

Rind disease of sugarcane, caused by *Pleocyta sacchari* (Mass) Petr. et Syd. is widespread in Brazil and other sugarcane growing countries (Leu *et al*[6]). During the 1975-76 harvest, in Brazil, severe losses from the disease occurred in some varieties following drought and delays in the processing of harvested cane. The physiology of the pathogen in relation to its optimum growth and sporulation was investigated. Spores, under optimum conditions, occur in clusters or masses.

The disease was described in Iraq (Mansour[7]). It is commonly found in seed-cuttings cultivated late in the planting season (Autumn) and causes substantial reduction in germination. It also occurs on standing over-riped cane in late winter.

P. sacchari was isolated from mixed juice collected from the crushers of the factory (Mansour *et al*[8]). *In vitro,* the fungus caused a drastic decrease in pol (% juice) and increase in reducing sugars within 20 days.

Saprophytic behaviour of *P. sacchari* has not been elucidated before, thus, the present investigation was initiated to study some aspects of its saprophytic behaviour and survival.

MATERIALS AND METHODS

Isolates

Isolates SC 28 and SC 53 of *P. sacchari*, unless otherwise stated were used throughout and maintained on potato dextrose agar. Cultures were obtained from Cane Diseases Laboratory, Misan Sugar Enterprise, Iraq.

Physiological Studies

Growth on different agar media

Linear growth rate, growth density and sporulation (at 28°C) were measured on five agar media, i.e., potato dextrose (PDA), malt extract, Richard's solution, oat meal and crude cane juice agar (2%), prepared in the usual way.

Effect of the addition of yeast extract on the growth

The fungus grows poorly on synthetic media. Attempts were therefore made to improve fungal growth through the addition of a source of growth factors. Difco yeast extract (Y.E.) was chosen for this purpose. A basal medium consisting of the constituents of Richard's solution except the original carbohydrate (sucrose) was prepared. Two levels of sucrose; 1.0 and 5.0% were then added and 0.1% Y.E. was added to each of the two levels. Controls free of Y.E. were provided. Media were distributed in 100 ml Ehrlenmayer flasks in 25 ml quantities and autoclaved. Flasks were inoculated with 4-mm agar culture disks and incubated at 28°C. After 15 and 30 days, growth was filtered off and dry weight of mycelium estimated.

Assimilation of carbon compounds

Assimilation of different carbon sources was examined in a nutrient solution containing 4.0 g carbon/liter and the other constituents of Richard's solution supplemented by 0.1% g Y.E. The amount of carbon was made to equal 1% glucose. The carbon source was added in the respective amount to

the sterile basal solution. The medium was distributed aseptically in 100 ml Ehrlenmayer flasks and reautoclaved instantaneously. The flasks were inoculated with 4 mm diameter culture disks and incubated at 28°C. The dry weight of mycelium was determined after 15 and 30 days.

Assimilation of nitrogen compounds

Assimilation of nitrogen compounds was examined in a nutrient solution consisting of 1.385 g n/l and the constituents of Richard's solution (10 g sucrose) supplemented by 0.1% yeast extract. The amount of nitrogen added was equal to 1% potassium nitrate. The N-sources were added to the sterilized basal solution. Likewise, flasks were inoculated and readings of the mycelium weight were made after 15 and 30 days incubation at 29°C.

Growth on cane juice

Crude juice of 12-month old NCo310 was distributed in 25 ml quantities in 100 ml Ehrlenmayer flasks and autoclaved at 15 lb/sq. in for 15 min. Flasks were inoculated with 4-mm agar culture disks and incubated at 28°C. Dry weight of mycelium was estimated after 15 and 30 days using 5 replicate flasks after each period.

Competitive saprophytic ability (CSA)

Isolate SC 28 of *P. sacchari* was used to measure CSA using the agar plate modification of the Cambridge method (Rao[9], Wastie[12]). The inoculum was prepared by growing the fungus for 1 month on supplemented fine bagasse (60 ml potato decoction containing 2% sucrose + 10 g fine sugar cane bagasse). Seven inoculum-soil (field soil) mixtures were prepared containing 100, 90, 70, 50, 30, 10, or 0% inoculum (W/W). Ten grams of each mixture were then dispersed in 10 ml of sterile, melted cooled water agar in a petri dish (9 cm diameter). Four mm diameter disks of the fungus cut out of these plates immediately, 24 and 48 h after the agar had solidified. The disks were transferred to fresh plates of PDA (4 to a plate), with 5 replicate plates per mixture. The number of disks (out of twenty) which gave rise to the inoculant fungus was counted after 5 days incubation at 28°C.

The Cambridge method (Garrett[3]) for determining CSA was also used. Mixtures of inoculum (grown as above) and soil were prepared containing 0, 10, 30, 50, 80, 90 and 100% of the inoculum. Five plastic tins (13 cm diam) were filled with each of these mixtures. The substrate to be buried in the mixture was 20 g cane air dry bagasse. The mixtures were sprayed with sterile water and then the tins were covered with their lids and left at about 28°C for 50 days. The buried bagasse was transferred to plastic pots, (13 cm diam), containing autoclaved river silt-peat moss mixture (4:1 W/W) and thoroughly mixed with the top 7 cm layer. Two surface sterilized 1-bud NCo310 cane cuttings were planted in each pot and kept in the greenhouse at 25-30°C. Tap water was used for watering the plants. The presence of *P. sacchari* in the buried bagasse was determined by the effect on the % germination after

30 and 60 days and the appearance of rind disease symptom on the cuttings after 60 days. The percentage germination and appearance of disease symptoms on cuttings from any mixture measured the degree of colonization of the substrate (Garrett[3]).

Tolerance to antibiotics produced by soil-microorganisms

Following Wastie's[12] method, PDA plates were inoculated centrally with a few milligrams of field soil (cultivated with sugar cane for 13 years) and covered with a 3 x 4 cm sheet of sterile cellophane. A 4 mm diam disc cut from an agar culture of the fungus was placed on the cellophane above the soil, either immediately after preparing the soil-inoculated plates, or after incubation for 24 and 48 hrs. Control plates without soil were inoculated in a similar manner. Ten replicate plates were prepared for each treatment.

In another experiment, 10 g of field soil were suspended in 90 ml sterile water, heated to 80°C for 10 minutes and then quickly cooled. The soil suspension was shaken vigorously and allowed to settle. Loopfuls of the supernatant were streaked across the center of PDA plates which were then incubated for 48 hrs: Two 4 mm diam. disks cut from an agar culture were placed 3 cm on each side of the streak. The width of inhibition zones was measured after 6 days incubation at 28°C, i.e. when the fungus would grow no farther towards the streak.

Decomposition of cellulose

Decomposition of two cellulosic substrates, filter paper and sugar cane bagasse were examined. The method using filter paper was described by Garrett[4]. The flasks were inoculated with 4 mm diam. disks cut from the margin of an agar culture and incubated at 28°C for 60 and 100 days. The filter paper was then removed, rinsed gently in three changes of distilled water and dried at 60°C to constant weight. The percentage loss in dry weight of filter paper due to cellulose decomposition in each of 8 replicate flasks was calculated.

Decomposition of bagasse was examined by a technique similar to that described by Sabet *et al*[11]. One gram of air-dried fine bagasse was placed into each of 16,100 ml Ehrlenmayer flasks and wetted with 25 ml Richard's solution containing 0.1% Y.E. The flasks were autoclaved, inoculated and incubated as above. The bagasse was then removed from each flask, washed gently in distilled water, dried at 60°C for 3 days, and left exposed in the laboratory to regain air dry weight, for another 2 days. The percentage loss in weight in 8 replicates was determined after each period.

Saprophytic survival

Survival of *P. sacchari* was examined in pure cultures grown on a supplemented substrate. Twenty grams of sorghum grains wetted by 50 ml potato decoction containing 2% sucrose in each of 250 ml Ehrlenmayer flasks were used. Flasks were autoclaved at 15 lb/sq in., inoculated with a 10 cm agar

culture disks and left at room temperature. The viability of the fungus was examined monthly by aseptically transferring the colonized grains onto fresh PDA plates.

Surviving structures produced on different agar media were examined. Sclerotia were killed and fixed in formalin acetic acid-alcohol (FAA). They were then dehydrated, infiltrated with paraffin and embedded in blocks of the same material. Twenty to 25 micron thick sections were prepared and the stained with safranin and aniline blue (Jensen[5]).

RESULTS

Physiological Studies

Growth on different agar media

Linear growth rate (Table 1) of five isolates of *P. sacchari* showed little variation either on the same medium or on different media. However, growth density variability differed indicating exacting nutritional requirements. Sporulation was more or less the same on the different media. Sclerotia were formed after 20 days on PDA, potato dextrose, oat meal and cane juice agar. Chlamydospores were formed on Richard's solution agar.

TABLE 1. Growth of *Pleocyta sacchari* on different agar media expressed as linear growth rate* (G.R.) and growth density** (G.D.)

Isolates	Agar media									
	P D A		Malt extract		Richard		Oat meal		Cane juice	
	G.R.	G.D.	G.R.	G.D.	G.R.	G.D.	G.R.	G.D.	G.R.	G.D.
SC 18	27.5	++++	25.6	++	26.0	++	22.0	+++	28.0	++
SC 26	20.9	++++	20.1	++	20.0	++	20.7	++++	28.5	++
SC 28	19.0	++++	22.1	++	12.5	+	20.0	++++	19.5	++
SC 53	22.9	+++	19.0	+	24.1	++	22.0	+++	19.2	++
SC 54	19.6	++++	14.3	++	18.1	++	21.3	+++	21.8	+++

* *mm/day*

** *In 6-day-old cultures.*

Effect of the addition of yeast extraction on the growth

The fungus grew very poorly on Richard's solution at 1 and 5% sucrose but much better when supplemented by Y.E. Growth on Y.E. was higher at 5% sucrose than at 1% and growth continued up to 30 days (Table 2).

TABLE 2. Effect of 0.1% yeast extract on the growth of *Pleocyta sacchari* (SC 28)

Basal solution +		Dry weight of mycelium (mg) after	
Sucrose %	Y.E. %	15 days	30 days
1	0.0	77.93± 9.23	130.9 ±16.53
5	0.0	78.13±12.08	121.1 ± 0.09
1	0.1	152.8 ±18.50	93.13± 2.92
5	0.1	278. ±18.46	309.9 ±18.2

Assimilation of carbon compounds

The results (Table 3) showed that after 15 days incubation, sucrose seemed to be the most suitable C-source followed by galactose, maltose, lactose and glucose. After 30 days, these sources were still the best compounds to support the growth. Glycerol, a polyhydric alcohol, was the least suitable. The fungus grew on the basal solution containing Y.E. but not on the basal solution only. On suitable C-sources, thick, grey to olive growth was produced.

TABLE 3. Effect of different carbon sources on the growth of *Pleocyta sacchari*, isolate SC 28.

Carbon sources	Dry weight of mycelium/mg after	
	15 days	30 days
Glucose	94.8	81.4
Galactose	133.0	91.6
Sucrose	141.6	92.8
Lactose	100.6	80.9
Maltose	103.6	85.4
Glycerol	87.8	79.0
Basal solution + Y.E.	39.5	41.3
Basal solution	0.0	0.0

Assimilation of nitrogen sources

The results (Table 4) showed that after 15 days of incubation, peptone was the most favorable source of nitrogen followed by potassium nitrate and ammonium sulphate. These substances were also the top best in readings taken after 30 days. Urea was markedly less suitable. The fungus grew poorly on basal solution containing Y.E. but not on basal solution only.

TABLE 4. Effect of different nitrogen sources on the growth of *Pleocyta sacchari*, isolates SC 28

Nitrogen sources	Dry weight of mycelium/mg after	
	15 days	30 days
Potassium nitrate	139.2	94.8
Ammonium sulphate	120.0	89.0
Peptone	151.4	140.0
Urea	76.0	96.0
Basal solution + Y.E.	78.0	89.0
Basal solution	0.0	0.0

Growth on cane juice

Results (Table 5) showed that after 10 and 20 days, the fungus grew very profusely on crude cane juice. Thick mycelium with dark olive color was produced. Sclerotia were formed after 20 days.

TABLE 5. Effect of crude cane juice on the growth of *Pleocyta sacchari*

Isolates	Dry weight of mycelium (mg.) after	
	10 days	20 days
SC 18	1307	1426
SC 26	1272	1372
SC 28	1203	1335
SC 53	1379	1476
SC 54	1305	1359

Competitive saprophytic ability

The agar-plate method (Table 6) show that *P. sacchari* had a high CSA when the inoculum-soil mixture disks were transferred immediately. However, after incubation for 24 hr the CSA apparently decreased. After 48 hr at lower inoculum ratios (0 and 10), CSA was still very low; at intermediate ratios it became high again, but still below 0 hr. and at 100% inoculum it continued to drop. The apparent drop in CSA at 24 hr may be due to the antagonistic effect of soil micro-organisms and air borne contaminants.

In the original Cambridge procedure, CSA was similar to the agar plate method when disks were transferred immediately regarding the % germination after 30 and 60 days (Table 7). Disease symptoms were observed after 60 days on cuttings cultivated in a 100% inoculum. However, isolation revealed the presence of *P. sacchari* in cuttings cultivated in 10 to 100% inoculum.

TABLE 6. Competitive saprophytic ability of *Pleocyta sacchari* estimated by the agar-plate modification of the Cambridge method

% inoculum (in inoc. – soil mixtures)	Time inoc. – soil mixtures were incubated before transfer of disks		
	0 h	24 h	48 h
	Colonized (%)		
0	0	0	0
10	67	0	0
30	74	7	60
50	74	47	60
70	87	34	67
90	87	14	40
100	94	34	20

TABLE 7. Competitive saprophytic ability of *Pleocyta sacchari* estimated by the Cambridge method and expresses as % germination of NCo 310 variety

% inoculum (in inoc. – soil mixtures	% germination after / days	
	30	60
0	100	100
10	75	75
30	75	75
50	75	75
80	50	50
90	50	50
100	0.0	0.0

Tolerance to antibiotics produced by soil microorganisms

P. sacchari did not grow on the soil-inoculated agar plates (Wastie method) but grew on plates without soil. In the streak method, growth was inhibited by bacteria and actinomycetes developing along tne streaks. The mean width of inhibition zone after 6 days of incubation was 8.44±0.94 mm.

Pectolytic activity

P. sacchari was grown on Richard's solution supplemented by 0.1% Y.E. After 7, 15, and 21 days of incubation at 28°C the cultures were filtered and presence of protopectinase in the filtrates was tested. Potato disks (1 cm diam

and 0.1 mm thick) were soaked in the filtrate in 50 ml flasks, covered with polyethylene, and incubated at 28°C for 24 h. No desintegration of the slices was observed.

Cellulose decomposition

P. sacchari actively decomposed filter paper (Table 8) and isolate SC 53 was highly active between 60 and 100 days. The increase in weight of filter paper shown with isolate SC 28 after 60 days, could be attributed to the growth of the fungus with little or no decomposition. The weight of uninoculated control was unchanged.

TABLE 8. Decomposition of filter paper by two isolates of *Pleocyta sacchari*

Isolates	Loss (–) or gain (+) in wt. of filter paper (%) after	
	60 days	100 days
SC 28	+5.51±1.35	–14.75±2.35
SC 53	–9.95±3.35	–60.07±3.92
Control	———	———

The fungus grew well on the bagasse supplemented by the inorganic basal solution. The loss in air-dry weight (Table 9) caused by both fungal isolates used may be attributed to cellulose decomposition, which was the main carbon source in the substrate. Although isolate SC 53 showed an exceptionally high cellulose-decomposing ability of filter paper, the two isolates decomposed bagasse with a high constant rate during the two incubation periods.

TABLE 9. Decomposition of bagasse by two isolates of *Pleocyta sacchari*

Isolates	% loss in air – dry weight of bagasse after	
	60 days	100 days
SC 28	9.27 ± 1.57	22.74±1.10
SC 53	10.77 ± 1.12	24.89±0.87

Spores and sclerotia were formed on both substrates and viability of the fungus continued for more than 16 months.

Saprophytic survival in pure cultures

P. sacchari survived in pure cultures grown on sorghum grains up to 17 months. On agar media, i.e. P.D.A., potato-dextrose oat meal and cane juice agar, the fungus formed sclerotia-like structures. They were small in size, with a round to spherical in shape (Fig. 1) and measured 0.5 to 1.5 mm in diam.

They were formed on the surface of the media within 20 to 25 days at 28°C. On supplemented bagasse they were formed within 15 to 20 days at room temperature. Formation of sclerotia is favored by continuous light. At first, dirty white powdery dots, 1.5 to 2 mm diam., appeared scattered on the surface. Later on, sclerotium developed at the center of the dot as black colored rounded to spherical body. In addition, sclerotia may be formed embedded in the mycelium mat (smaller sclerotia).

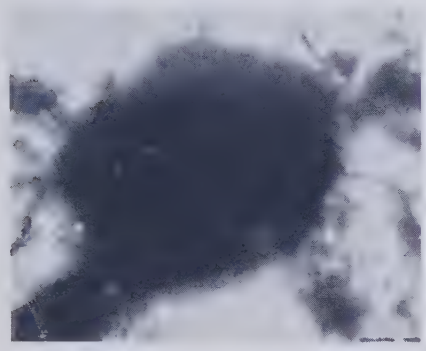

FIGURE 1. Saprophytic behavior of *P. sacchari* cause of rind disease of sugarcane

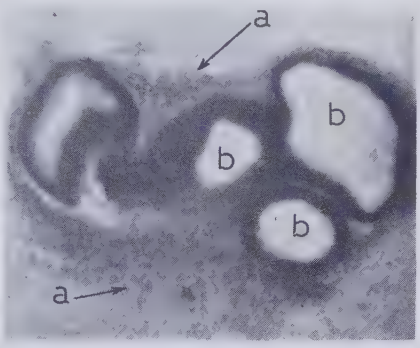

FIGURE 2. Saprophytic behavior of *P. sacchari* cause of rind disease of sugarcane

Sclerotia were taken out of the culture, cleaned from mycelium and sterilized in hydrogen peroxide (20%) for 1 minute, then washed in sterile water. They were then transferred onto fresh PDA for germination at 28°C. They readily germinated after 2-3 days.

Microscopic examination of prepared sections showed that each sclerotium is made up of a number of pycnidia (2-5) and coated with a thick short-cell mycelium layer (Fig. 2). Each pycnidium is surrounded by a separate pseudo-parenchy-matus wall (Figs. 3a & b). Spores are formed and extruding from the mouth of the pycnidium.

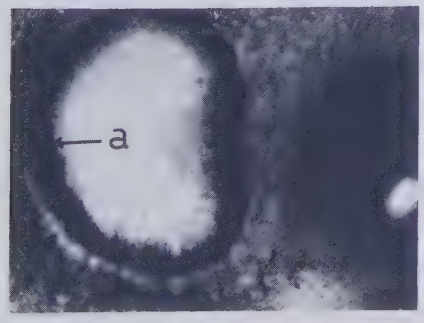

FIGURE 3a. Saprophytic behavior of *P. sacchari* cause of rind disease of sugarcane

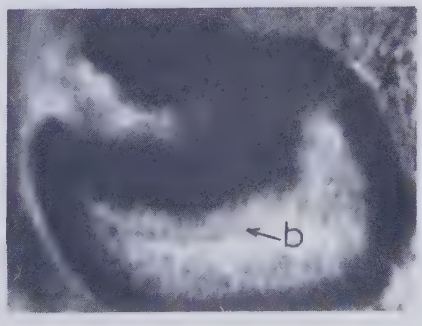

FIGURE 3b. Saprophytic behavior of *P. sacchari* cause of rind disease of sugarcane

DISCUSSION AND CONCLUSION

Physiological studies indicated exacting nutritional requirements for *Pleocyta sacchari.* Growth was considerably enhanced by the addition of 0.1% yeast extract to the basal mineral solution containing 1% sucrose and 1% potassium nitrate. The fungus can utilize a number of simple mono and disaccharides as source of carbon but polyhydric alcohol was not suitable. Peptone was the most favourable nitrogen source. The fungus did not grow in the absence of suitable carbon and nitrogen compounds in a basal solution free of Y.E. but grew vigorously on crude cane juice.

Using the agar-plate modification of the Cambridge method, it was shown that *P. sacchari* had a high CSA when the disks were transferred immediately. However, incubation of the soil-inoculum mixtures for 24 hrs before cutting the disks and transferring them to final plates, markedly lowered the CSA. Incubation for 48 hrs. raised it again, but it was still below that measured immediately. The after incubation effect is ascribed to the antagonestic effect of soil microorganisms and air borne contaminants. This agreed with the high sensitivity showed by the fungus to antibiotics produced by soil microorganisms and inhibition by bacteria and actinomycetes.

These results prove the suggestions of Garrett[4] that fast spore germination, fast growth, the ability to produce many enzymes and antibiotics, and tolerance to antibiotics produced by other microorganisms were factors likely to make an organism a competitive saprophyte. Using the original Cambridge method, the fungus colonized the buried substrate in a manner similar to that which occurred after 0 h using the modified method. Sabet and Khan[10] classified 5 cotton-root-infecting fungi, colonizing over 85% of agar plates at 10% inoculum, as highly competitive.

Sabet *et al*[11] found that *Cephalosporium maydis* had a little CSA, since it was unable to develop from any of the impregnated soil-inoculum disks. Accordingly, *P. sacchari* could be classified as a moderate competitive saprophyte.

P. sacchari actively decomposes cellulose of filter paper and bagasse. This is significant as far as the industrial qualities of stored bagasse are concerned.

Optimum conditions for sporulation of *P. sacchari* were described by Liu *et al*[6]. Spores occur in spore masses either on agar media or sterile stem tissues. The present investigation showed that the fungus produces sclerotium — like structures which may have not been described before. Cross-sections of these structures showed that they consist of several pycnidia. They are similar to that produced by *Hindersonina sacchari* Butler and described as sclerotia Abbott[1]. In addition, these structures are also described as pycnidiophores which are fruit-bodies containing pycnidia Ainsworth *et al*[2]. Both fungi, *P. sacchari* and *H. sacchari*, belong to the group Sphaeropsidales which is characterized by producing pycnidia. Thus the structures produced by *P. sacchari* could be described as sclerotia.

Since *P. sacchari* is a moderate competitive saprophyte and a high cellulose decomposer, it is suggested that it survives in nature growing on plant tissue remains or in the form of sclerotia and chlamydospores. Such assumption has to be investigated.

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COMPORTAMIENTO SAPROFITICO DE *PLEOCYTA SACCHARI* EL AGENTE CAUSAL DE LA ENFERMEDAD DE LA CORTEZA DE LA CAÑA DE AZUCAR

I. M. Mansour

RESUMEN

El hongo *Pleocyta sacchari* crece y germina bien en una gran variedad de medios de cultivos. Se indican exactamente los requisitos de nutricion en la variacion de velocidad y de densidad de crecimiento y germinacion. Se forma sclerotia por el hongo en PDA, harina de maiz y jugo de caña. Se aumento considerablemente el crecimiento en los medios de cultivos de mineral por la adicion de 0.1% de extracto de la levadura.

La habilidad saprofitica competitiva (HSC) que fuera estimada, tanto por placas de agar como por los metodos de Cambridge, sugieren que *P. sacchari* es un saprofitico competitivo moderado.

P. sacchari descompone activamente la celulosa del papel de filtro y bagaso de la caña de azucar. Esto es significativo en lo que consierne a la calidad industrial del bagazo almacenado. No se produce pectolytic filtrado en los cultivos por el hongo. Es susceptible a antibioticos que se producen por los microorganismos en suelo.

Se forma sclerotia en medios de cultivos y substratos de la celulosa con un numero de pycenidia. El hongo sobrevivira mas que 16 meses en los medios de cultivos puros y sembrado en los granos de sorgo esteril y suplementado y en los substratos de la celulosa.

Se sugirieron que el hongo sobrevivira en la naturaleza creciendo en las sobras de las tejidos de las plantas o en la forma de sclerotia y de clamidoesporas.

UPTAKE AND TRANSLOCATION OF CERTAIN SYSTEMIC FUNGICIDES BY SUGARCANE PLANT AND THEIR CONTROL OF RIND DISEASE

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ABSTRACT

Fundazol (Methyl–1–butylcarbamoyl–2–benzimidazol–carbamate) and Bayleton (1 – (4-chloro-phenoxy) – 3, 3– dimethyl – 1–(1 H –1 2,4 – triazol–1–yl) – 2– butanone), at 10 ppm (as formulated), and KWG 0519 15 DS 151 A at 50 ppm, completely inhibited, *in vitro,* the growth of *Pleocyta sacchari,* the causal fungus of rind disease.

Juice extracted from different organs of sugarcane plants grown in Fundazol-treated nutrient solution and/or soil was fungitoxic. The highest toxicity was detected in juice of roots growing from seed cuttings and the lowest in juice of seed pieces. Bayleton and KWG were highly phytotoxic and inhibited or suppressed root production and development. This resulted in marked suppression of uptake.

In pot experiments, the three fungicides at 500 ppm/unit wt dry soil added at planting time, completely controlled rind disease on seed pieces. Fundazol stimulated root production and germination.

High toxic effect was detected after 120 days in sterile or infested soil treated with each of the three fungicides separately, either planted or unplanted with sugarcane.

INTRODUCTION

Systemic fungicides were used to control different rots of sugarcane seed pieces. Benomyl (Methyl – 1 – butylcarbamoyl – 2– benzimidazol carbamate) formulated as 50% wettable powder under the name Benlate has been found to be a satisfactory substitute to the mercury based fungicides against pineapple disease (Hilton *et al*[3] Thompson[11], and Ricaud[9]). Benlate at 0.5 lb per 100 gal water was recommended for cold dip treatment and 0.25

lb/100 gal water for the short hot treatment at 50°C for 30 minutes or 52°C for 20 minutes[3] (Hilton *et al* 1971). The same concentration can be used for the long hot treatment at 50°C for 2 hours. Dipping single-budded cuttings, artificially inoculated with *Ceratocystis paradoxa,* in 0.1% Benlate effectively controlled the pineapple disease and stimulated germination and growth (Wang *et al*[12]). In pot experiments, rind disease caused by *Pleocyta sacchari* was effectively controlled on seed cuttings at 1000 ppm Benlate (as formulated)/unit wt dry soil added at planting (Mansour[5]). Application on Benlate in amounts of 140 and 560 g a.i./ha in tests against yellow spot increased cane sugar content but was not adequately effective (Egan[1]). Maximum germination of 2-budded cuttings, dipped in fungicide solution 15 minutes before planting, was obtained with 1000 ppm Demosan (1,4-dichloro – 2,5 – dimethoxybenze) (Muthusamy[8]). It also reduced the incidence of pineapple disease in ungerminated setts and gave better results than Agallol (Methoxyethyl mercury chloride). Cuttings were dipped for varying periods in 0.05% Benomyl or 0.5% Agallol solution with the first node above the solution level (Sulladmath *et al*[10]). Two-mm thick disk-shaped samples were cut from the inner core of cuttings at four -depths, each time, and bioassayed for fungitoxicity to *Ceratocystis paradoxa, in vitro.* Benomyl was superior to Agallol and gave a maximum inhibition of 28.5% after 5 minutes dipping.

Uptake and translocation of Benlate, Vitavax – Captan and/or Thiram (UniRoyal's 5-6 dihydro –2– methyl –1,4–oxathin –3– Carbozanilide – Captan and/or Thiram) by sugar cane plants were studied in fungicide-treated nutrient solution, juice of different plants organ bioassayed (after 30 and 60 days) for fungi-toxicity showed that Benlate was present in seed pieces at 100 ppm, in roots at 50 ppm and in shoots at 1000 ppm (as formulated). Toxicity of Vitavax-Captan was detected only in seed pieces and roots at 500 ppm, while Vitavax-Thiram was detected in seed pieces at 100 ppm and in roots at 10 to 100 ppm (in higher levels root production was suppressed). After 60 days, toxicity of Vitavax compounds was not detected in any of the plant organs. In treated soil, Benlate was detected in seed pieces and shoots at 3000 ppm and in roots at 1000 ppm after 30 days. After 60 days, toxicant accummulated in shoots at 2000 ppm. Toxicity of both Vitavax compounds was not detected at any level. In both nutrient solution and soil treatment, Benlate stimulated root production and growth. Vitavax compounds were markedly phytotoxic and severely inhibited root production and development.

MATERIALS AND METHODS

Fungicides

Benomyl (Methyl – 1 – butylcarbamoyl – 2 – benzimidazol – carbamate) formulated as 50% wettable powder under the name Fundazol Bayleton (25% WP), 1 – (4 – chloro-phenoxy) – 3,3–dimethyl –1–(1 H-1–2,4–triazol –1–yl) –2– butanone, and KWG 0519 15 DS 151 A (Bayer) were used throughout this investigation.

Effect on mycelial growth

Effect of the fungicides on mycelial growth of *Pleocyta sacchari,* the causal fungus of rind disease of sugar cane, was investigated. Fungicides were incorporated in melted, potato dextrose agar (PDA), cooled to 50°C at the rate of 10, 50, 100, 500 and 1000 ppm w/v. Five replicate plates, 9 cm in diameter, containing 20 ml of the treated medium were inoculated in the center with a 4 mm agar culture disks. Fungicide-free plates prepared in the same way served as control. The percentage reduction in colony diameter was determined after 6 days incubation at 28°C.

Effect of autoclaving

Effect of autoclaving on the activity of the fungicides was determined. Two treatments in which fungicides were incorporated in PDA either before or after autoclaving were prepared using 10, 50 and 100 ppm. Autoclaving was effected at 15 lb/in^2 for 15 minutes. Five replicate plates containing 20 ml of the treated medium were inoculated as above and incubated at 28°C. Percentage reduction on colony diameter was estimated after 6 days.

Uptake and translocation

Uptake and translocation was determined using two methods: dipping cuttings of NCo 310 sugar cane variety in fungicide – treated nutrient solution, and planting seed pieces in treated soil.

(a) Dipping: Each of the fungicides was applied alone in autoclaved Hoagland and Synder's for salts nutrient solution (Mclean and Cooke[7]), at the rate of 10, 50, 100, 500, 1000 and 2000 ppm (w/v). Nutrient solution alone served as a control. Solutions were prepared in large plastic pots, 40 cm diameter, covered with glass sheets. Twenty 5-budded cane cuttings were dipped in an acropetal direction, in each of the solutions with the first node above the solution level and the upper cut surface sealed with paraffin. They were then kept at 25-30°C for 30 days and 60 days.

(b) Soil treatment: River silt-peat mixture (4:1 w/v) was treated with each of the fungicides separately at the rate of 500, 1000, 2000 and 3000 ppm/kg air dry soil prior to planting in plastic pots (13 cm diameter, capacity 1.5 kg air dry soil. The amount of fungicides to be applied to each pot was mixed first with about 100 g of the soil mixture and then with the top 5-7 cm layer of the pot soil. Two one-bud cuttings were planted in each of 10 replicate pots. Fungicide-free treatment was provided and all pots were left under green house conditions at 30-35°C for 30 and 60 days.

Bioassay of plant tissues

After each of the two periods of dipping and/or planting, 10 cuttings or seedlings were taken out from each treatment and thoroughly washed in running water and left for one hour to dry. Cane cuttings and/or pieces, roots (growing from the cuttings) and primary shoots were cut separately to small pieces and ground in a mortar. Juice of ground tissues was obtained through

a hand fruit juicer. Since results showed that no change in the fungitoxicity of the three fungicides was observed by autoclaving, all juices were autoclaved. This is in agreement with the previous work on benomyl (Gray and Sinclair[2]). Autoclaved strips 1.5 x 3 cm. of filter paper were dipped in the autoclaved juice and aseptically placed on the surface of 5 PDA 9 cm diameter plates. Three cm apart, on the opposite side, a 4 mm agar culture disk of *P. sacchari* (a sensitive indicator) was planted. After incubation for 5 days at 28°C, fungitoxicity of juice induced by filter paper strips of *P. sacchari* was estimated.

Control of rind disease

Isolate 28 of *P. sacchari,* causal fungus of rind disease of sugar cane, was used for soil infestation. Inoculum was prepared as described by Mansour[6] on supplemented sugar cane bagasse. Inoculum was mixed with the autoclaved soil (river silt-peat moss mixture) at the rate of 30 g/pot (13 cm diameter, capacity 1.5 kg air dry soil) prior to planting. Fungicides to be applied to the infested and/or sterile soil, were added at the rate of 500, 1000, 2000 and 3000 ppm/kg air dry soil and mixed with the 7 cm top layer of each pot soil before planting.

Persistence in soil

Fungitoxicity of treated soil was examined by a technique similar to that of Hine *et al*[4]. A culture of *P. sacchari* was used as an indicator. Treated soil disks were prepared by spreading 10 g autoclaved air dry in a sterile petri dish and impregnating it with 10 ml of molten, sterile water agar cooled to 45°C. Disks 10 mm in diameter were cut out after 4 hours and each transferred to the side of each of five PDA plates. To the opposite side, 3 cm apart, an agar culture disk of *P. sacchari* was transferred. After 6 days of incubation at 28°C, the antagonistic effect induced by the soil disk was estimated. Persistence was examined in the treated sterile or infested soil either unplanted or planted with NCo 310 variety.

RESULTS

In Vitro Tests

Effect of mycelial growth

Fundazol, Bayleton, and KWG 0519 15 DS 151 A at all concentrations caused 100% reduction in colony diameter of 3 isolates of *P. sacchari* except KWG at 10 ppm which showed 72-99%. When the ungrowing disks were re-examined for viability on fresh PDA 8 days later, Fundazol had a fungicidal effect at all levels used, and Baylon had a fungistatic effect at 10 and 50 ppm fungicidal effect at 100 to 1,000 ppm. KWG showed a fungistatic effect at 50 ppm and fungicidal effect at 100 to 1,000 ppm.

Uptake And Translocation

Nutrient solution — fungicide treatment

Fundazol was detected in cuttings only after 30 days at 2000 ppm (Table 1). It was detected in roots at all concentrations used and was completely inhibiting mycelial growth between 500 and 2000 ppm after 30 days, and between 100 and 2000 ppm after 60 days. In shoots, after 30 days, it was detected between 500 and 2000 ppm with a highly antagonistic effect. After 60 days, it was detected at the same levels, but the lower effect, may be as a result of the increased growth of shoots. In the nutrient solution, it was detected in all levels with a declining effect between 30 and 60 days. Bayleton was not detected in any of the plant organs at any concentrations. It was detected in the nutrient solution, containing 100 to 2000 ppm, between 30 and 60 days with a declining effect. KWG was detected only in seed cuttings at 2000 ppm with a weak effect. It was detected in the nutrient solution containing 500 to 2000 ppm between 30 and 60 days. Both Bayleton and KWG were phytotoxic and suppressed root production and germination at 500 to 2000 ppm.

Soil treatment

In both sterile and infested soil, Fundazol was detected in roots at all concentrations with increasing effect in infested soil after 60 days (Table 2). This may be due to the increased concentration of toxicant in a comparatively small volume of roots in infested soil.

In contrast, it was detected in shoots grown in sterile soil treated with 2000 to 3000 ppm. This may be due to the more absorption by the relatively activated roots in sterile soil. It was detected in cuttings specially after 60 days. Bayleton and KWG were highly phytotoxic and suppressed root production at all concentration in both sterile and infested soils. Germinating buds gave stunted shoots, which later dried. Toxicants were not detected in shoots of both treatments and detected in cuttings treated with 1000 to 3000 after 60 days.

Effect of soil treatment on sugar cane growth

In both sterile and infested soils planted with NCo 310 cuttings Fundazol stimulated root production, germination and shoot growth compared with the control (Table 3). Both Bayleton and KWG at all concentrations inhibited or suppressed root production but raised germination in infested soil. Stunted shoots were produced and soon wilted and dried.

Control of rind disease

In pot experiments, rind disease on seed pieces were completely controlled by applications equivalent to 500 ppm/kg air dry soil of each of the three fungicides separately added at planting. After 60 days, isolation from seed pieces showed the absence of *P. sacchari,* the rind disease pathogen.

Persistence in soil

In pot experiments, soil with different treatments was bioassayed for fungitoxicity every 40 days up to 120 days (60 days after plants had been dug up). The results (Table 4), show the mean width of inhibition zone caused by different soil treatments. Maximum inhibition was induced by Fundazol followed by Bayleton and KWG. The latter was not detected in any of the soils treated with 500 ppm. Bayleton was not detected in sterilized soil treated with 500 ppm but detected at the same level in unplanted soil. This may be due to complete absorption of the toxicant by the plants. After 120 days, very high fungitoxicity of Fundazol-treated soil, at all levels used, was detected at 120 days. KWG persisted only in soils treated with 1000 ppm and above.

TABLE 1. Uptake and translocation of the systemic fungicides, applied in a nutrient solution by sugar cane plants as detected by juice fungitoxicity to *Pleocyta sacchari* on PDA

Treatments		Fungitoxicity							
		After 30 days				After 60 days			
Fungicides	Concentrations PPM	Juice of				Juice of			
		Nutrient Sol.	Cuttings	Roots	Shoots	Nutrient Sol.	Cuttings	Roots	Shoots
Fundazol	10	++	–	++	–	++	–	++	–
	50	++	–	++	–	+	–	+	–
	100	++	–	++	–	+	–	+++	–
	500	+++	–	+++	++	+++	–	+++	+
	1000	+++	–	+++	++	+++	–	+++	+
	2000	+++	+	+++	++	+++	–	+++	+
Bayleton	10	–	–	–	–	–	–	–	–
	50	–	–	–	–	–	–	–	–
	100	+	–	+	–	+	–	–	–
	500	++	–	*	–	+	–	*	*
	1000	+++	–	*	*	++	–	*	*
	2000	+++	–	*	*	+++	–	*	*
KWG	10	–	–	–	–	–	–	–	–
	50	–	–	–	–	–	–	–	–
	100	–	–	–	–	–	–	–	–
	500	+	–	*	*	+	–	*	*
	1000	++	–	*	*	++	–	*	*
	2000	++	+	*	*	++	–	*	*
Control	0.0	–	–	–	–	–	–	–	–

* *Suppressed roots and/or shoots*

– *No effect*

+, ++, +++ *indicates antagonistic, highly antagonistic and completely inhibiting respectively.*

TABLE 2. Uptake and translocation of the systemic fungicides applied in soil, by sugar cane plants, as detected by juice fungitoxicity to *Pleocyta sacchari* on PDA

Treatments		Sterile Soil						Infested Soil					
		Plant organs** and effect after						Plant organs** and effect after					
Fungicides	Concentrations	30 days			60 days			30 days			60 days		
		C	R	S	C	R	S	C	R	S	C	R	S
Funda-	500	–	+	–	–	++	+	–	+	–	–	++	–
zol	1000	–	+	+	–	++	+	–	++	–	+	++	–
	2000	–	++	+	–	++	+	–	++	–	+	+++	+
	3000	+	++	+	–	++	+	+	++	+	+	+++	+
Bayl-	500	–	*	–	–	–	–	–	*	–	–	*	–
eton	1000	–	*	–	–	*	–	–	*	–	–	*	–
	2000	–	*	–	–	*	–	–	*	–	–	*	–
	3000	–	*	–	–	*	–	–	*	–	–	*	–
KWG	500	–	*	–	–	*	–	–	*	–	–	*	–
	1000	–	*	–	+	*	–	–	*	–	–	*	–
	2000	–	*	–	+	*	–	–	*	–	+	*	–
	3000	–	*	–	+	*	–	–	*	–	+	*	–
Control	0.0	–	–	–	–	–	–	–	–	–	–	–	–

** *C, R and S indicate cuttings, roots and shoots, respectively*

* *Suppressed roots*

–, +, ++ *and* +++ *indicate no effect, antagonistic, highly antagonistic and completely inhibiting, respectively.*

TABLE 3. Effect of soil treatment with the systemic fungicides on growth characteristics of NCo 310 cane variety

Treatments		Sterile Soil				Infested Soil			
Fungicides	Concentration ppm	% germination after 30 days	% germination after 60 days	Developing of Roots	Developing of Shoots	% germination after 30 days	% germination after 60 days	Developing of Roots	Developing of Shoots
Fundazole	500	95	100	A	A	65	80	N	N
	1000	100	100	N to A	A	75	90	N	N
	2000	100	100	N to A	A	65	95	N	N
	3000	100	100	N to A	A	85	95	N	N
Bayleton	500	90	95	DW	DW	75	85	DW	DW
	1000	100	100	DW	DW	95	95	S	DW
	2000	60	65	S	DW	60	75	S	DW
	3000	50	60	S	DW	65	65	S	DW
KWG	500	90	90	DW	DW	95	100	DW	DW
	1000	90	100	DW	DW	100	100	DW	DW
	2000	100	100	S	DW	75	85	S	DW
	3000	100	100	S	DW	85	100	S	DW
Cont.	0.0	95	100	N	N	25	50	N	S to N

S, N, and A, indicate suppressed, normal and activated roots and/or shoots, respectively, Dw indicate stunted wilted roots or shoots.

TABLE 4. Persistence of the systemic fungicides in soil as examined by fungitoxicity to *Pleocyta sacchari*, after 120 days.

Soil treatments			Fungicides and mean width of inhibition zone, mm after 6 days		
Infestation	Planting	Levels of fungicides	Fundazol	Bayleton	KWG
Infested	Planted	500	28.0	14.0	0.0
		1000	25.8	19.4	16.0
		2000	24.5	20.0	19.0
		3000	26.2	27.5	22.2
Sterile	Planted	500	24.6	0.0	0.0
		1000	24.7	15.5	19.4
		2000	27.0	20.0	22.6
		***			
Sterile	Unplanted**	500	25.8	12.7	0.0
		1000	25.6	16.0	14.0
		2000	24.4	20.0	20.0
		***			

* *Infested with* P. sacchari
** *Watered regularly*
*** *3000 ppm was not incorporated*

DISCUSSION AND CONCLUSION

Fundazol (benomyl) at 10 ppm completely inhibited growth of *Pleocyta sacchari.* Bayleton at the same concentration and KWG 0519 15 DS 151 A at 50 ppm completely inhibited the growth but had only fungistatic effect. The activity of the three fungicides was not changed by autoclaving.

Fundazol in treated nutrient solution and/or soil, was readily absorbed by and translocated in sugar cane plants in a manner similar to that shown by Mansour[5], but with comparatively higher rates. Toxic substance seemed to accummulate in roots and gradually translocated to shoots. Toxicant gradually declines in shoots as a result of growth and increasing tissue volume. As shown by Mansour[5], intake of toxicants was markedly increased in the nutrient solution than in soil. This may be due to soil physical properties and leaching. Fundazol, obviously, stimulated root production and germination with no detrimental effect up to 3000 ppm.

Bayleton and KWG in both treatments, were highly phytotoxic and severely inhibited or suppressed root production. They increased germination, but developed shoots soon wilted and dried.

In pot experiments, rind disease on seed cuttings was completely controlled by applications equivalent to 500 ppm/kg air dry soil, of each of the three fungicides separately added to the planting time.

High toxic effect of the three fungicides was detected in treated soils after 120 days. Fundazol showed the maximum effect followed by Bayleton and KWG.

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LA ABSORCIÓN Y LA TRANSLOCACIÓN DE CIERTOS FUNGICIDAS SISTÉMICOS POR LA PLANTA DE LA CAÑA DE AZÚCAR Y SU CONTROL SOBRE LA ENFERMEDAD DE LA CORTEZA

I. M. Mansour

RESUMEN

Fundazol (Methyl-1-butycarbamoyl -2- benzimidazol-carbamate) y Bayleton (1- (4- chloro-phenoxy) -3, 3- dimethyl -1- (1-H-1-2, 4-triazol -1- yl) -2- butanone a 10 ppm (como formulada) y KWG 0519 15 DS 151 A, a 50 ppm inhibición completamente, in vitro, el crecimiento de *Pleocyta sacchari,* el hongo causal de la enfermedad de la corteza.

El jugo exprimido de los órganos de plantas de caña de azucar que crecieron en la solución nutritiva o en el suelo tratado con Fundazol fue fungitóxico. La mayor toxicidad se encontró en el jugo de raices que crecián de las semillas "seed cuttings y la mas baja en el jugo de semillas "seed pieces". Bayleton y KGW fueron altamente fitotoxicos e inhibieron o suprimieron la producción y el desarrollo de la ráiz. Esto resultó en una disminución marcada de la absorción.

En los experimentos de tiestos, los tres fungicidas a 500 ppm/ unidad del suelo seco añadido al tiempo de la siembra controlaron completamente la enfermedad de corteza de las semillas. Fundazol obviamente estimuló la producción y la germinacion de la raiz.

Un alto efecto fitotoxico se detectó despues de 120 diás en suelo estéril o infestado y tratado con cada uno de los tres fungicidas separadamente, ya fuera sembrado o sin sembrar con caña de azucar.

PROTECTION OF SUGARCANE AGAINST RED ROT DISEASE WITH *FUSARIUM MONILIFORME*

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ABSTRACT

Protection of sugarcane with *Fusarium moniliforme*, the causal agent of Fusarium stem rot, against *Colletotrichum falcatum*, causal organism for red rot, and the factors responsible for this phenomenom were studied. The results showed that a previous inoculation of sugarcane stalks with *F. moniliforme* would reduce the rate of rotting caused by *C. falcatum*. The threshold for inducing the function of protection by *Fusarium* was found to be around 4 hours after inoculation. Evidence indicated that obstructions in the vascular bundles produced by *Fusarium*, which mechanically prevent migration of red rot spores through the vessel, is probably the main cause of cross protection.

INTRODUCTION

Red rot, caused by *Collectotrichum falcatum* Went., is one of the principal and widespread diseases of sugarcane in Brazil. Varietal resistance test against this disease by artificial inoculation showed CB 45-3 and Co 331, the most cultivated commercial varieties in Northeast Brazil, to be susceptible. Reactions of these two varieties from artificial inoculations with *Fusarium moniliforme*, the causal agent of Fusarium stem rot, were usually less severe than those of *C. falcatum*.

Field investigations of the incidence of red rot on CB 45-3 and Co 331 at thirty one sugarcane mills in Pernambuco, Paraiba and Rio Grande Do Norte (Liu[7]) showed that rotting caused by the disease was less severe on these two varieties than when they were artificially inoculated. Infection through borer tunnels occurs very commonly, and is always associated with infection by *F. moniliforme*. A *Fusarium-Collectrichum* disease complex with obscure symptoms of rotting without the distinct white patches, which are specific for red rot, is usually produced. The extent of the disease complex development is usually confined to a limited area in the stalk indicating that protection against red rot disease occurs if the infection site is first infected with *F. moniliforme*.

It has been reported that varieties of sugarcane inoculated with *F.*

moniliforme, followed by the *C. falcatum* inoculation in the same corkborer openings 8 days later, gave in, general, more resistant ratings for stalk rotting than inoculation with *C. falcatum* only (Anon[3]).

Tests were conducted at the Sugarcane Experiment Station, Carpina, Northern Regional Co-ordination Service, IAA/Planalsucar, during 1977 and 1978 to determine whether first inoculating sugarcane stalks with *F. moniliforme* would reduce the amount of rotting caused by *C. falcatum* and to understand the mechanism responsible for the reduction in severity of red rot disease to *F. moniliforme.*

MATERIALS AND METHODS

Sugarcane Varieties

Stalks of the varieties CB 45-3, Co 331, Co 997 and CP 51-22, ten to 12 months of age were used for these studies. The reactions of the varieties to red rot and Fusarium stem rot by artificial inoculations were listed as follows:

Variety	Red rot	Fusarium stem rot
CB 45-3	Intermediate susceptible	Intermediate resistant
Co 331	Highly susceptible	Intermediate resistant
Co 997	Intermediate susceptible	Intermediate resistant
CP 51-22	Intermediate resistant	Intermediate resistant

Preparation of Inoculum

1. Spore Suspensions

Ten to 12 day-old cultures of *F. moniliforme* and *C. falcatum* on the cane juice agar were used for preparation of spore suspensions. Concentrations of inocula were determined by spore counts with a hemacytometer. Fresh inocula were prepared and kept in the refrigerator overnight for inoculations the following morning.

2. Non-sporulating Mycelium Suspension

Three day old cultures of *C. falcatum* on cane juice agar were thoroughly washed with tap water to remove the spores as well as the aerial mycelia. The remaining subterranean mycelia in each Petri dish were fragmented in a Waring Blendor jar with 100 ml. of distilled water for about 60 seconds. No spores were observed when the inocula were examined under the microscope.

Artificial Inoculation

1. Protection against C. falcatum with F. moniliforme

To determine whether first inoculating stalks with *F. moniliforme* would reduce the rate of rotting caused by *C. falcatum,* test were made on 10 to 12 month old standing canes of CB 45-3, Co 331 and CP 51-22 in the field. Spore suspensions of the fungi were introduced into holes made in the central

were covered with adhesive tape. Five stalks of normal size, without borer infection or cracking, were inoculated with each of the following treatments:

Fm	– inoculated with *F. moniliforme*
Cf	– inoculated with *C. falcatum*
Fm + Cf	– inoculated with a mixture of *F. moniliforme* and *C. falcatum* in equal parts.
Fm – Cf_{3d}	– inoculated with *F. moniliforme* followed by *C. falcatum* inoculation in the same corkborer openings 3 days later
Fm – Cf_{6d}	– as above but inoculated with *C. falcatum* 6 days later
Fm – Cf_{9d}	– as above but inoculated with *C. falcatum* 9 days later

Fifty-four to 66 days following inoculation, the stalks were split and the results recorded. Ratings for red rot and *Fusarium* stem rot infections were classified on a scale of 0 to 9 according to a "Guide for assigning ratings to red rot infection"[2], in which, 0 means no infection and 9, total or extremely severe rotting of the stalk.

2. Threshold for Inducing Protection

Test to determine the threshold for a previous inoculation in stalks with *F. moniliforme* to induce protection against red hot caused by *C. falcatum* were made by inoculating setts in the laboratory. Healthy 4-node setts, taken from the central and top portions of 10 to 12 months old stalks of Co 997, were used. Both ends of the setts were disinfected with Benlate (0.2%) to give protection from infection by pineapple disease *(Ceratocystis paradoxa).* Inoculations were made in the central part of the setts with the same procedure described for the inoculations of standing cane. Ten setts were inoculated with each of the following treatments:

Cf	– inoculated with *C. falcatum* at a conc. of 2.9×10^6/ml.
Fm	– inoculated with *F. moniliforme* at a concentration of $2{,}9 \times 10^6$/ml.
Fm + Cf	– inoculated with a mixture of equal parts of the above pathogens
Fm – Cf_2	– inoculated with *F. moniliforme* followed by *C. falcatum* inoculation in the same corkborer openings 2 hours later.
Fm – Cf_4	– as above, but inoculated with Cf 4 hours later.
Fm – Cf_8	– as above, but inoculated with Cf 8 hours later.
Fm Cf_{12}	– as above, but inoculated with Cf 12 hours later.
Fm Cf_{24}	– as above, but inoculated with Cf 24 hours later.

In an earlier study, inoculated setts were often contaminated with rind disease *(Pleocyta sacchari).* It made the results almost impossible to read. Rind disease is very common in our fields. It has been observed that the disease does not damage cane that is growing normally. However, the fungus is commonly

present in the nodal tissues of growing cane through natural infection, where it remains dormant while the cane is growing. However it can cause severe damage when growth ceases as in the case of severe drought or over maturity. To prevent the development of rind disease in the test setts, the 4-node setts were planted vertically with the lowest nodes buried in moist sand in flats after inoculation. Setts watered daily. Rind disease rarely occurred on these setts. Twelve to 14 days following inoculation, the setts were split and the results recorded.

3. Mechanisms of Cross Protection

Single node setts of Co 997, about 12 cm in length, was cut so there was 10 cm of stalk above the leaf scar and 2 cm below, were used for studying the mechanism of protection in sugarcane with the Fusarium stem rot and red rot pathogens. Inoculum of either spore or mycelium suspensions were smeared over the entire surface of the top ends. After inoculation, the top ends were covered with parafilm or aluminum foil to maintain enough moisture for establishing infection of the diseases. The basal ends were disinfected with Benlate (0.2%) to protect against infection by pineapple disease. Ten setts were inoculated for each of the following treatments:

0 – inoculated with *F. moniliforme,* followed by *C. falcatum* inoculation on top ends 12 hours later.
1 – inoculated with *F. moniliforme,* followed by *C. falcatum* inoculation on top ends 12 hours later, after a thin layer, about 1 mm in thickness, of Fusarium infected tissue was removed.
2 – as above, with 2 mm of tissue removed.
4 – as above, with 4 mm of tissue removed.
8 – as above, with 8 mm of tissue removed.

Check – inoculated with *C. falcatum* without previous inoculation of *F. moniliforme.*
Concentration of Fm and Cf – 2.9×10^6/ml.

Setts were planted vertically in moist sand after inoculation. Twelve to 14 days following inoculation the setts were split and results recorded.

RESULTS

Protection Against *Collectrichum* in sugarcane with *Fusarium*

The results (Table 1) show that the rotting of stalks by *C. falcatum* was significantly reduced if they were previously infected with *F. moniliforme.* No protection was found in any of the test varieties when inoculations with both pathogens were made simultaneously in the same corkborer openings.

Threshold for Inducing Protection

The threshold for inducing protection against red rot disease from a previous inoculation with *F. moniliforme* was found to be around 4 hours (Table 2). A pre-inoculation of *F. moniliforme* followed by *C. falcatum* inoculation 2 hours later, did not give protection. However, Co 997 inoculated with *F. moniliforme* followed by *C. falcatum* inoculation in the same corkborer openings at 4, 8, 12, and 24 hours later, showed more resistance to stalk rotting than inoculations with *C. falcatum* only, Fm + Cf or Fm – Cf_2. The results were statistically significant. (Table 2).

Mechanism of Protection

1. Evidence of Conidia Migration in Vascular Bundles

To demonstrate that the conidia of *C. falcatum* migrate in vascular bundles and establish new points of infection in internodes beyond the one in which they gain entrance, single node setts were used as described earlier. It has been observed that the white patches which indicate the establishment of red rot infection, usually occurred in upper, central and sometimes in basal portions of those setts 12 days after inoculation with spore suspension (Table 3). Similar white patches were only found in the upper portions of internodes inoculated with a mycelium suspension, since there were no spores present to migrate through the vascular bundles and establish new points of infection.

TABLE 1. Effect of *Fusarium moniliforme* infected tissue, subsequently inoculated with *Colletotrichum falcatum* on the development of rotting in sugarcane stalks.

Treatment*	Rating of disease infection						Incubation period (days)
	Co 311		CB 45-3		CP 51-22		
	rate	sym. exp.	rate	sym. exp.	rate	sym. exp.	
FM	3.0	fsr	3.2	fsr	3.2	fsr	66
Cf	7.6	rr	5.8	rr	6.0	rr	60
Fm + Cf	6.8	rr	6.0	rr	5.6	rr	60
Fm – Cf_{3d}	3.0	obs	3.2	obs	4.0	obs	63
Fm – Cf_{6d}	3.2	obs	3.6	obs	3.4	obs	60
Fm – Cf_{9d}	3.0	obs	3.6	obs	3.0	obs	54

* *Five stalks were inoculated for each treatment.*

Fm – *inoculated with* F. moniliforme.
Cf – *inoculated with* C. Falcatum.
Fm + Cf – *inoculated with a mixture of* F. moniliforme *and* C. falcatum *in equal parts*
Fm + Cf_{3d} – *inoculated with* F. moniliforme, *followed by* C. falcatum *inoculation in the same corkborer openings 3 days later.*
Fm – Cf_{6d} – *as above, but inoculated with Cf 6 days later.*
Fm – Cf_{9d} – *as above, but inoculated with Cf 9 days later.*
Rate – *rating based on an average of 5 replications.*
Sym. exp. – *symptoms expression: fsr – showing typical symptoms of Fusarium stem rt; rr – showing typical symptoms of red rot with distinct white patches; obs – showing obscure symptoms without white patches, could be Fusarium stem rot or Fusarium-Colletotrichum complex.*

TABLE 2. Threshold for a previous inoculation in stalks of Co 997 with *F. moniliforme* for inducing protection against red rot caused by *C. falcatum* in sugarcane.

Treatment	Rating of stem rot on ten 4-node setts*										Symptoms	
	1	2	3	4	5	6	7	8	9	10	av	
Cf	5	6	6	6	6	6	7	7	7	7	6.4a**	+
Fm	3	3	3	3	4	4	4	4	4	4	3.6b	–
Fm + Cf	4	4	6	6	6	6	7	7	7	7	6.2a	+
Fm – Cf_2	6	6	6	7	7	7	7	7	7	7	6.8a	+
Fm – Cf_4	3	3	4	4	4	4	5	6	6	6	4.5b	–
Fm – Cf_8	3	3	3	4	4	4	4	4	4	5	3.8b	–
Fm – Cf_{12}	3	3	3	3	4	4	4	4	5	5	3.8b	–
Fm – Cf_{24}	3	4	4	4	4	4	4	4	5	5	4.1b	–

* – *10 setts of Co 997 were used for each treatment.*

Cf – *inoculated with* C. falcatum *at a concentration of 2.9 x 10^6/ml.*
Fm – *inoculated with* F. moniliforme *at a concentration of 2.9 x 10^6/ml.*
Fm + Cf – *inoculated with a mixture in equal parts of two pathogens mentioned above.*
Fm – Cf_2 to Fm – Cf_{24} – *inoculated with* F. moniliforme, *followed by* C. falcatum *inoculation in the same corkborer openings, 2, 4, 8, 12 and 24 hours later.*
\+ – *showing typical red rot symptoms with characteristics white patches.*
– – *without white patches.*
av – *means of 10 replications.*
** – *means not followed by a common letter are significantly different at the 1% level (Duncan's multiple range).*

TABLE 3. Occurrence of red rot symptoms on single node setts inoculated with spore and mycelial suspensions of *C. falcatum*, after the Fusarium infected tissue was removed.

Thickness of Fusarium infected tissue removed (mm).	White patches on setts inoculated with Cf by			
	Spore suspension		Mycelial suspension	
	Portions with white patches	Intensity	Portions with white patches	Intensity
0		–		–
1	top	++	*	–
2	top	++		–
4	top, central	+++		–
8	top, central	+++		–
Check	top, central	+++	top	++

0 = *inoculated with* F. moniliforme, *followed by* C. falcatum *inoculation on top ends 12 hours later; Number 1, 2, 4 and 8 represent thickness in mm of tissue removed from top of setts 12 hours after inoculation with* F. moniliforme *and prior to inoculation with* C. falcatum; * – *few necrotic spots at central and basal portions of internodes.*

2. Evidence of obstruction by Fusarium in vascular bundles that confine the migration of spores of C. falcatum

Bourne[6] stated that the *Fusarium* infection spreads most rapidly in the vascular bundles. Ayson[5] found the mycelium in the vascular bundles four inches from the point of inoculation after 68 days. To determine whether the presence of *F. moniliforme* mycelium in the vascular bundles would limit the migration of spores of *C. falcatum* in vessels, tests were made with the single node sett technique. The results (Table 3) showed that when single node setts inoculated with *F. moniliforme,* followed by *C. falcatum* inoculation at the top ends 12 hours later, white patches were not formed either in setts inoculated with spores or mycelium. However, when different thickness (1 to 8 mm) of *Fusarium* infected tissues were removed, followed by *C. falcatum* inoculation on the cut ends, various intensities of white particles were observed in internodes only on setts inoculated with spores. The more *Fusarium* infected tissues removed the more white patches were detected in the internodes. Obstructions of the vascular bundles by the *Fusarium* fungus, which prevents migration of the red rot spores through the vessels, is probably the principal factor in the protection by *C. falcatum* in Northeast Brazil.

DISCUSSION

A summary of the findings from these studies (Table 4), illustrates that the most important factor in inducing protection by *F. moniliforme* against infection by *C. falcatum* in sugarcane is probably the blockage of the vascular bundles by the *Fusarium* fungus. It has been demonstrated that protection of sugarcane stalk from infection with *C. falcatum* occurs when sugarcane tissues are previously infected by *Fusarium* Laboratory observations with the hanging drop technique showed that germination of *F. moniliforme* spores began at around 3 hours after incubation at 28°C, while 98% germination was observed after 6 hours. Germination of spores in a mixture of both pathogens was normal. The 4 hours time lapse required to produce protection against *C. falcatum* coincided with the time required for germination of *F. moniliforme,* although this 4-hour threshold for germination is dependent on environment conditions. Since the *Fusarium* spores germinate with a period of 3 to 6 hours on sugarcane tissues, infection occurs, causing obstructions in vascular bundles, so spores of *C. falcatum* are not able to migrate through the vessels (Table 4-6). There was no protection (Table 4-7) where the obstructions were removed.

Abbott[1] stated that two types of resistance to red rot are recognized: physiology of the living cells of the plant which prevents or suppresses the development of the parasite within the host tissues; and morphological, which is related to the presence or modification of structures in the plant tissue which mechanically prevent or retard infection and development of the parasite. Atkinson[4] found that Co 290 has a large number of continuous vascular bundles through the nodes, which permit ready migration of spores through the stalk. The evidence in these studies, showing *Fusarium* obstructions which mechanically prevent or restrict the migration of *C. falcatum* in vascular bundles, is considered as morphological resistance. This morphological resistance associated with *Fusarium* infection is believed to play an important role in the

biological control of red rot in our sugarcane fields. Investigations of stem rots in Northeast Brazil (Liu[7]) revealed that more than 97% appeared to be from the *Fusarium-Collectotrichum* complex which produced limited rotting in sugarcane stalks because the tissues first became infected with the ubiquitous *Fusarium* spores through borer tunnels. During cultivation of susceptible varieties, such as CB 45-3 and Co 331 on a large commercial scale for more than 20 years in Northeast Brazil, no severe damage by red rot was ever recorded. The protection by *Fusarium* is believed to be responsible for this.

TABLE 4. Summary of results related to the protection of sugarcane inoculated with *Fusarium moniliforme* (Fm) and *Colletotrichum falcatum (Cf)*

Treatment	Red rot symptoms produced	Obstruction*	Migration**
1. Inoculated simultaneously with a mixture of Fm and Cf spores	+	–	+
2. Inoculated with Fm spores, followed by Cf spores inocu. in same holes 2 hrs later (Fm spores had not germinated)	+	–	+
3. Inoculated with Fm spores, followed by Cf spores in same holes 4 or more hours later	–	+	–
4. Inoculated with Cf spores on healthy single node setts (CK-1)	+	–	+
5. Inoculated with Cf mycelium on healthy 1-node setts (CK-2)	+	–	–
6. Inoculated with Cf spores on Fusarium infected tissues	–	+	–
7. Removed Fusarium infected tissues followed by Fm mycelium inocu.	+	–	+
8. Removed Fusarium infected tissues followed by Cf mycelium inocu.	–	–	–

* = *Obstruction in vascular bundles by Fusarium infection at time of inoculation with Cf.*
** = *migration conidia of Cf in vascular bundles*
– = *negative.*
\+ = *positive*

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PROTECCION DE LA CAÑA DE AZUCAR CONTRA LA ENFERMEDAD DE PODREDUMBRE ROJA CAUSADA POR *FUSARIUM MONILIFORME*

H. P. Liu, C. A. Wismer, S. L. Wang y M. C. C. Leite

RESUMEN

Se hicieron estudios sobre la proteccion de la caña de azucar con el hongo *Fusarium moniliforme*, el agente causal de la podredumbre de tallo *(Fusarium)* y contra el hongo *Collectotrichum falcatum*, agente causal de la podredumbre roja y los factores responsables de este fenomeno. Los resultados indican que una inoculacion (previa) de tallos de caña de azucar con el hongo *F. moniliforme* podria reducir la velocidad de la podredumbre causada por *C. falcatum*. El umbral para inducir la funcion de proteccion por *Fusarium* resultó ser aproximadamente 4 horas despues de la inoculacion. La evidencia indica que las obstrucciones en los haces vasculares producidas por *Fusarium* que impide mecanicamente la emigracion de las esporas en los haces vasculares, es probablemente la funcion principal en la proteccion cruzada.

COMMERCIAL CONTROL OF LEAF SCALD DISEASE BY THERMOTHERAPY AND A CLEAN SEED PROGRAMME

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ABSTRACT

Thermotherapy and a clean seed program are being used to control leaf scald disease on a commercial scale, for the first time in the world. The disease has been found on many farms in the Burdekin district of Australia since late 1976, but should be reduced to very low levels by 1982. The methods are described by which the healthy cane is produced, and propagated on isolated plots and on cane farms, before commercial plantings are made.

INTRODUCTION

Hot water treatment is used in Australia to free sugarcane planting material of chlorotic streak and ratoon stunting diseases. Of the other important diseases in Australia, only leaf scald *(Xanthomonas albilineans)* offered any possibility for control by the thermotherapuetic treatment of planting material.

Steindl[6] eliminated leaf scald disease from one-eye setts by soaking them in water at ambient temperatures for up to 48 hours prior to long hot water treatment (LHWT) at 50°C for three hours. The disease was not eliminated by LHWT alone. In South Africa, Bailey[1] also failed to eliminate leaf scald from diseased setts with LHWT of 50°C and 52°C for up to two hours.

Since the early 1970s, Steindl's method, referred to locally as the cold-soak/LHWT method, has been adopted as a Bureau of Sugar Experiment Station practice when transferring small amounts of cane from one environment where leaf scald is endemic, to another (Hughes[3]). No breakdown of the method has occurred, as far as is known. As an added bonus, there were strong indications that the pre-treatment water soaking made the cane more resistant to LHWT, resulting in better germinations.

The method appeared to be worth trying on a commercial scale when the opportunity arose.

Prior to 1976, leaf scald disease had been recorded in all districts in Australia, except in Burdekin. Susceptible varieties were grown there extensively, e.g. Trojan in the 1940s and 1950s, and Q63 since the 1960s. In November 1976, leaf scald

symptoms were discovered in the Burdekin district in Q63, a variety in which the disease may remain latent for long periods before expression of symptoms.

Up to May 1979, the disease had been located in more than 40 per cent of the farms in the district, although only a few blocks were infected heavily enough to show any yield loss. In retrospect, it appears that leaf scald was present for at least five years prior to its discovery.

The Burdekin district has approximately 850 cane farms harvesting up to 3.8 million tons of cane from up to 32,000 ha. The major variety for the last 10 years has been Q63, which first exceeded 50 per cent of the crop in 1972, and reached 68 per cent in the 1977 crop.

During the leaf scald disease epidemic in Q63 in the Mackay district of Queensland during the early 1970s (Persley[4]), yield losses occurred in ratoon crops when the epidemic reached an advanced stage. Approval to grow Q63 commercially in Mackay was withdrawn at the end of 1973 and the last crops were harvested as second ratoons in 1976, prior to ploughout. (N.B). The Director of Sugar Experiment Stations has a statutory obligation to issue annual lists of varieties approved for planting in each sugar mill area. A major factor which he must consider when approving a variety is resistance to disease.).

Leaf scald disease has always been controlled in Australia by releasing resistant varieties for commercial use, by removing susceptible varieties from cultivation, and by selecting healthy plant sources. This method could not be followed *in toto* for the Burdekin district in the short term. The new and quite different approach involving thermotherapy was chosen. This was aided by the presence of Q96, a variety which was superior to Q63 in yielding ability, of intermediate reaction to leaf scald, and which was about to be approved for planting when leaf scald was discovered.

In early 1977, it was decided, in conjunction with grower and miller groups, to remove Q63 from the 1979 lists of varieties approved for planting in the Burdekin district. Under BSES disease control regulations, this meant that the last commercial crops of Q63 would be harvested in 1981 as second ratoons, and then ploughed out. Most of the leaf scald disease should be removed in this way also. The percentage of Q63 in the Burdekin crop will plummet from the maximum of 68 percent reached in 1977, to zero in 1982.

It was also decided to approve Q96 as a commercial variety in 1979, and to build up healthy stocks of Q96 through a large scale cold-soak/LHWT program. Associated with this would be a designated plant sources scheme in order to limit the possibility of reinfection of the healthy cane.

During the 1977, and 1978 plantings, restrictions were placed on the amount of the susceptible Q63 which could be planted from what appeared to be healthy sources, and cane grower were encouraged to plant leaf-scald-resistant varieties such as Q80, 90, 97 and 98. It is hoped that a reasonable percentage of resistant canes can be maintained in the future.

FIELD PROGRAM

Production of disease-free seed cane in isolated plots

A system of primary and secondary-increase plots was established in the Burkedin district in 1977, along lines similar to those initiated in the Bundaberg district of southern Queensland to control Fuji disease (Egan[2]). A moderate degree of isolation was required for these plots to guard against reinfection by leaf scald disease. A nucleus of Q96 was planted each year in the primary plot with one-eye setts given the cold-soak/LHWT, and using the previous year's treated cane as a source. The progeny was used to plant the secondary increase plots (SIPs) in the succeeding year, and the stubble was then ploughed out. Details of the areas planted each year are given in Table 1.

TABLE 1. Areas planted in Burdek in plots, and tons of Q96 and other varieties bought by farmers from plots

Area/tons	1977	1978	1979
Hectares Q96 planted, primary plot	1.2	1.1	1.1
Hectares Q96 planted, SIPs	20.4	22.1	24.3
Tons Q96 sold SIPs	—	2 030	1 895
Tons other varieties sold plots	1 954	2 485	2 120

Similarly for the SIPs, only the plant crop was used to provide cane growers with a nucleus of healthy Q96 plants (see Table 1 for tons supplied), and the stubble was then ploughed out. Hygiene in planting and harvesting operation was maintained carefully in these plots. In this way, Q96 planting material, free of both leaf scald and ratoon stunting diseases, was supplied virtually to all of the 850 cane farms in the district. The average amount was 2 tons per cane grower, but this varied considerably with farm size.

This program was initiated on a large scale in 1977 so the whole of the SIP planting, i.e. 20.4 hectares, also was made with one-eye setts given the standard cold-soak/LHWT. Germination was excellent despite the use of some immature cane.

The three cane Pest and Disease Control Boards operating in the Burdekin district are responsible for the production of disease-free planting material. They also arrange for the harvest and cartage of the plants to the cane farm, on the instructions of the cane grower.

Plants of the various leaf-scald-resistant varieties also are produced by these Boards for sale to cane grower. However, these clean cane plots do not need to be

isolated from commercial cane, and the cane to plant them is given an LHWT without the pre-soak in water. The average amount supplied each year is around 3 tons per cane grower.

The designated plant source scheme on farms

Although Q96 is listed as an approved variety, restrictions have been placed on the sources of planting material which may be used. In practice, the canegrower buys a nucleus of disease-free Q96 from the SIP *each year,* grows it on his farm in a designated site which must not be adjacent to Q63 fields, and uses this source for his commercial plantings in the succeeding year *only.*

Other approved varieties (Q80, Q90, Q97, Q98) are included in the designated plant source scheme also, but restrictions are less severe than for Q96. The purchase of a nucleus of disease-free cane each year from the SIP is encouraged, but there is no compulsion on the cane grower to do so. All plant sources are inspected at least once by Cane Pest and Disease Control Board staff, and any which showed disease (e.g. RSD, chlorotic streak) would be discarded.

RESULTS AND DISCUSSION

This is the first report of therapeutic measures being used to control leaf scald disease in commercial plantings. The scheme is possible partly because of the statutory powers on disease control which BSES possesses, the co-operation of canegrowers and sugar millers in the Burdekin district; and the Cane Pest and Disease Control Board system in Australia. In this case, the Ayr, Inkerman and Invicta Boards have operated the scheme under BSES supervision.

The first nucleus of Q96 plants was supplied to growers in 1978. This was planted in over 5,000 ha, approximately 60 percent of their total planting in 1979. Some leaf scald infection of Q96 probably will occur on farms in the short term since diseased fields of Q63 are still present throughout the district. However, the potential for transmission by mechanical and other means should decrease markedly as the diseased fields are ploughed out progressively. The scheme will face its most severe test over the next three years. Thereafter, the pressure on it will ease considerably. However, leaf scald disease probably cannot be eradicated from the district because of latency problems in some varieties and the probable presence of the disease in alternative hosts (Ryan[5]).

More research is needed to improve the commercial aspects of the method. Questions whether 2-eye setts or 3-eye setts can be used instead of 1-eye sett, whether whole stalks can be used and whether the 48-hour pre-soak in water can be shortened, or replaced by some other pre-treatment should be answered.

Control of RSD by LHWT was developed in Australia in the early 1950s. Over the 10-year period 1969-78, an average of 3,200 tons of cane has undergone LHWT each year. It has not been possible to eradicate RSD, but its incidence in

commercial crops can be held at very low levels by using inspected plants from recently-treated sources and by giving attention to hygiene. Quite susceptible varieties can be grown commercially with negligible or zero yield losses, provided these precautions are observed.

It is not claimed that an equally successful system has been developed for the commercial control of leaf scald disease, but it appears to be quite promising at this stage. There are differences between the pathogens and transmission methods for the two diseases, which could affect the outcome. Field experience over a period of several years will be necessary before any definite conclusions can be made.

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CONTROL COMERCIAL DE LA ENFERMEDAD DE LA ESCALDADURA POR TERMOTERAPIA Y POR UN PROGRAMA DE SEMILLAS SANAS

B. T. Egan y O. W. Sturgess

RESUMEN

La termoterapia y un programa de semilla sana han sido usados por primera vez en el mundo para controlar la enfermedad del escaldadura en escala comercial. La enfermedad se ha encontrado en muchas fincas en el distrito de Burdekin en Australia desde finales de 1976, pero deberia ser reducida a niveles muy bajos para el 1982. Se describen los metodos que se usan para producir y propagar las semillas sanas en las parcelas aisladas y en las fincas de caña antes de llevar a cabo siembras comerciales.

FEASIBILITY OF FUNGICIDAL CONTROL OF YELLOW SPOT

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ABSTRACT

The effectiveness of Benomyl sprays (270 g a.i./ha) for controlling yellow spot disease in the profusely flowering susceptible variety S 17 was evaluated under severe epiphytotic conditions. Sprays applied with mist blowers at intervals of 1, 3 and 4 weeks were compared. There were no differences between treatments at 3- and 4-week intervals. Although they did not control infection as well as weekly sprays, they were nearly as effective in giving a substantial increase in sucrose content. Spraying also improved juice purity but had no effect on cane yield at harvest early in the season.

There was a greater response to fungicide control in flowered than in vegetative canes at harvest time. In an industrial trial, four fungicide sprays with mist blowers at intervals of 5 weeks gave 57% control of infection and an increase in sugar yield of 1 to 2 tons/ha. Disease control was also effective when Benomyl was applied above the leaf canopy with ULV sprayers. Therefore, fungicidal control of yellow spot in sugarcane could be feasible and economic and can be a useful temporary measure to reduce losses in case of change of resistance in varieties, pending their replacement.

INTRODUCTION

Yellow spot disease caused by *Cercospora koepkei* Kruger of sugarcane occurs in several tropical countries. It can be severe in high rainfall areas, causing losses in both cane and sugar yields (Egan[5,6], Hughes and Ocfemia[7], Ricaud[11,12]).

So far, its control relies solely on the use of resistant varieties. This is, however, not entirely satisfactory; the recurrence of severe epiphytotics associated with changes in varietal resistance has been reported (Egan[5], Ricaud *et al.*[13,14]).

Attempts to control the disease by fungicide spraying have been reported (Egan[4], Prakasam and Satyanarayana[9], Ricaud[12]). Fungicide Benomyl was found to give good control but unfortunately several sprays are required.

The heavy losses assessed in variety S 17 in the recent epiphytotics in Mauritius have prompted further investigations on fungicidal control.

MATERIALS AND METHODS

Four field trials of each of the following were conducted:

(i) Two small-plot trials to determine the optimum interval for fungicide spraying.
(ii) A strip-type trial to assess the feasibility of control on an industrial scale.
(iii) A small-plot trial in which an ultra low volume sprayer was used for spraying over the top of the cane canopy to simulate aerial spraying.

The first three trials were conducted in 1978 and the fourth in 1979.

Plant material

The trials were carried out in 2nd or 3rd ratoon fields of the susceptible variety S 17, a high sucrose, profusely flowering variety adapted for early harvest in the super-humid zone of Mauritius (average annual rainfall is greater than 2500 mm), in which severe epiphytotics of the disease occur yearly since 1976.

Fungicide spraying

Benomyl (50% wp methyl 1-(butylcarbamyl) - 2 - benzimidazole carbamate) was used for spraying at the rate of 270 g a.i./ha. For the first spraying in each trial, Dithane M-45 (a co-ordination product of zinc ion and manganese ethylene-bisdithiocarbamate, 80% WP) was added to the spray suspension at the rate of 860 g a.i./ha. In the first 3 trials, mist blowers were used for spraying and the spray jet was directed towards the undersurface of cane leaves. One thousand liters of fungicide suspension in water, to which were added 80 ml of spreader sticker Triton B 1956 were applied per hectare.

In the fourth trial, Micron ULVA 8 ultra low volume sprayers (Micron sprayers Ltd. U.K.) were used and the sprays were drifted on the cane leaves from slightly above (0.2 m) the canopy. The fungicide was suspended in a 5% oil in water emulsion, applied at the rate of 25 1/ha. Sunspray 7 E (Sun Oil Co., Belgium) was used.

Spraying was started early February and continued in most treatments until the end of May or early June when infection started to subside. Weekly sprays were performed until the end of June.

Treatments and layout

In the 3 small-plot trials, the effectiveness of spraying at intervals of 1, 3 and

4 weeks was compared. Unsprayed plots served as control. A 4 x 4 latin square design was adopted, plot size being 6 rows x 10 m, two of which were non-experimental guard rows.

In the industrial trial, plots receiving 4 fungicide sprays at intervals of 5 weeks from mid-February to end of May were compared to unsprayed plots. A randomized block design with 4 replicates was used, each plot consisting of 16 rows x 96 m (i.e. 0.25 ha).

Data and statistical analysis

The effectiveness of treatment was assessed by determining disease build-up, cane yield and sugar content. The results on disease infection for the 1979 trial only are presented in this paper.

The incidence of flowering was less than 75% in the first 2 trials and data for infection and sugar content were collected separately for flowered and vegetative stalks. In the industrial trial where the incidence was greater than 85%, vegetative stalks were juvenile and stunted, therefore only flowered stalks were considered.

Disease build-up was monitored at intervals of 4 to 5 weeks from the beginning of February until August. Percent leaf area covered by yellow spot was assessed visually on leaves 1 to 10 on 10 stalks (or 2 x 10, if both flowered and vegetative assessed) taken at random from each replicate plot, as previously described (Ricaud[10]).

Cane yield was determined at harvest at 12 months. In the first 2 trials, the 2 middle rows were weighed for each plot. In the industrial trial 2 plots of 6 rows x 70 m. diagonally opposite each other in each strip, were cut and loaded separately in 2 crates which were weighed at the factory.

Stalk samples were analyzed at harvest for Brix, pol % cane, fiber %cane and reducing sugar content. These data were used to calculate IRSC (industrial recoverable sucrose % cane), purity, reducing sugar/sucrose ratio, tons sugar per hectare and tons profitable sugar per hectare. The latter is an index that takes into consideration harvest, transport and processing costs and penalizes cane with low sucrose when comparing yields from experimental plots.

In the first 2 trials, 10 stalks were sampled from each plot for analysis, for both flowered and vegetative stalks separately. Vegetative stalks were cut at their apex and flowering stalks at the base of the inflorescence. In the industrial trial, there were 3 sub-samples of 10 stalks per strip. The strips were subdivided approximately into 3 sub-plots from which the stalks were taken in random rows along a diagonal. In addition, for the industrial trial, analyses at harvest were also made on samples from the crates, using a core sampler. Each crate was analyzed separately and an aliquot from two cores was taken from each.

Pol %cane was also determined 1 and 2 months before harvest for the 3 trials, and 1 month after harvest on rows left standing, in the industrial trial.

For statistical analysis of infection build-up, the arcsine transformation was applied to the percentage values. In the latin square trials, data for flowered and vegetative canes were analyzed separately. For comparison of flowered vs vegetative, a pooled variance was calculated.

RESULTS

Optimum spraying interval

Climatic conditions in 1978 were very favorable for yellow spot infection. Similar results were obtained in both trials to determine the optimum interval for fungicide spraying, results for only one of them are presented.

There was no significant difference in the degree of control between spraying at 3- and 4-week intervals (Fig. 1). Both gave highly significant disease control compared to unsprayed plots but were less effective than weekly sprays which kept down infection to a very low level. Considering the cumulative infection over the period of observations (area under infection curves), spraying at 3- and 4-week intervals reduced disease build-up in flowered stalks by about 58% compared to 90% for weekly sprays. Differences among spray intervals in the flowered and vegetative stalks were highly significant in July. However, harvest data showed no significant differences in cane yield among the different treatments.

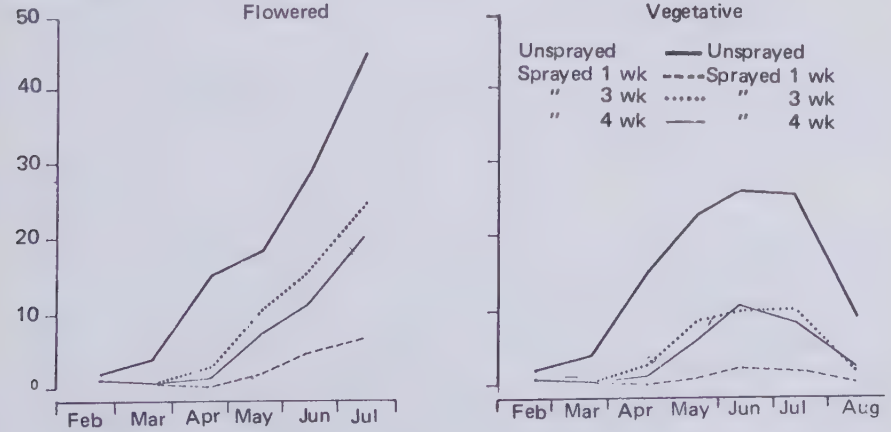

FIGURE 1. Progress of yellow spot infection in flowered and vegetative canes of variety S 17 in plots sprayed with Benomyl at intervals of 1, 3 and 4 weeks and in unsprayed plots

Fungicide spraying resulted in a substantial and significant increase in sucrose content in both flowered and vegetative stalks in June and at harvest in July (Fig. 2). There were no significant differences among the 3 spraying intervals, except for flowered stalks at harvest which showed significant (P 0.05) response to weekly sprays than to the other 2 spraying intervals.

Results in July showed that vegetative stalks responded less to spraying than flowered stalks, the minimum sucrose gain from any of the 3 treatments being 11.3% in vegetative compared to 23.0% in flowered stalks. This is due to the fact that the sucrose content of diseases canes in unsprayed plots in July is significantly higher in vegetative than in flowered stalks. In June, flowered and vegetative stalks were equally affected by the disease and the minimum gain from spraying was 28.2% and 23.4%, respectively.

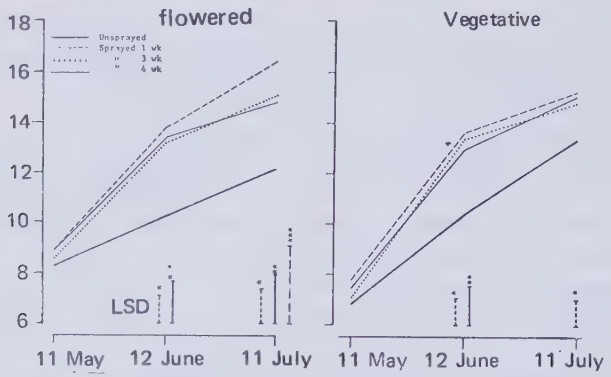

FIGURE 2. Increase in sucrose content in flowered and vegetative canes of variety S 17 resulting from the control of yellow spot by Benomyl sprays at intervals of 1, 3 and 4 weeks. (LSD – * P 0.05, ** P 0.01, *** P P 0.001)

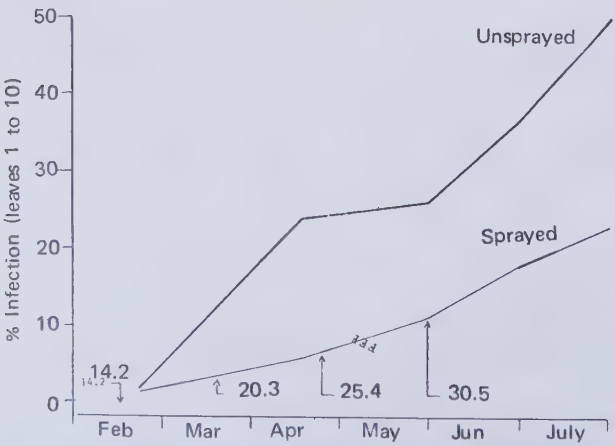

FIGURE 3. Control of yellow spot infection in variety S 17 on an industrial scale by four sprays of fingicide Benomyl. (Arrows indicate dates of spray).

Spraying also improved juice purity at harvest in flowered stalks although there were no significant differences among the 3 spray intervals. A mean of 92.8% was recorded for sprayed plots and 86.2% for unsprayed plots. In vegetative canes there were no significant differences among the three sprayed and unsprayed plots.

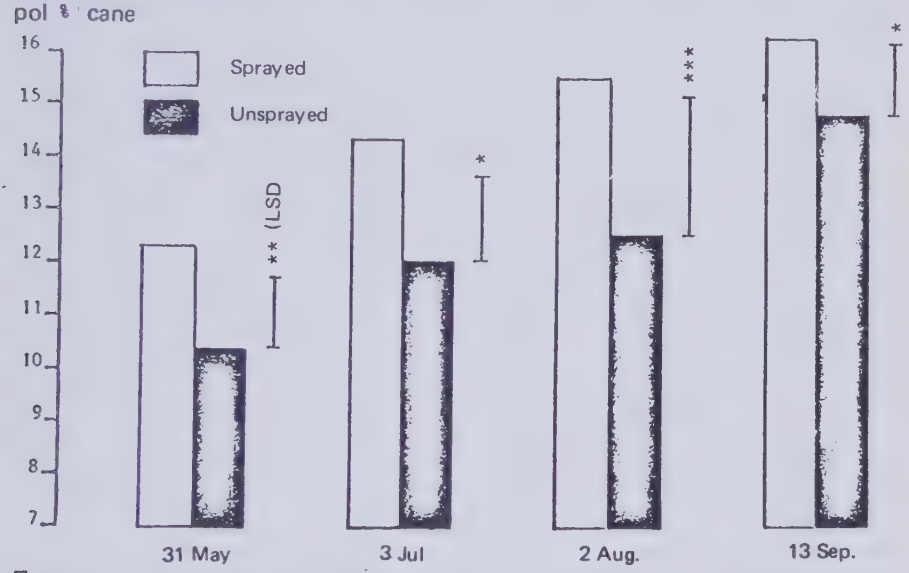

FIGURE 4. Increase in sucrose content in variety S 17 following control of yellow spot using Benomyl sprays in an industrial scale trial. (LSD – *P0.05, **P0.01, ***P 0.001)

Industrial trial

Spraying significantly reduced disease build-up in the industrial trial (Fig. 3). The cumulative infection level was reduced by 57%. Spraying, even at intervals of 5 weeks, resulted in a substantial and significant increase in sucrose especially at harvest in August and September (Fig. 4).

Harvest data are presented in Table 1. As in the previous trials, disease control did not give any increase in cane yield. However, the increase in sugar yield was at least 1 ton/ha. The higher response to spraying shown by the results with the field sampling technique was consistent with data from the analyses obtained one month earlier. In addition to the gain in sucrose, a marked and significant increase in juice purity was recorded, which was also reflected by the reducing sugar/sucrose ratio values.

Overhead spraying

Full results of the trial for investigating spray application from above

the cane canopy are not yet available. Preliminary data are shown here to indicate the feasibility of aerial spraying. Climatic conditions in 1979 were such that after an early start of infection, disease progress slowed down. Surveys in May revealed that fungicide application by the Micron ULVA sprayers gave significant disease control. Infection levels were 0.7%, 2.4% and 2.0% in plots sprayed at 1-, 3- and 4-week intervals respectively, as compared to 15.2% in the unsprayed control.

TABLE 1. Increase in sugar yield in variety S 17 as a result of yellow spot control using Benomyl sprays

	Sprayed	Unsprayed	LSD+	Gain%
Tons cane/ha	76.1	76.4	NS	–
IRSC++	11.8†	9.0	2. 4***	32
	11.0	9.7	1. 0*	11
Tons sugar/ha	8.98	6.88	1.60*	31
	8.37	7.38	0.93*	14
Tons profitable sugar/ha††	5.93	3.83	1.70**	55
	5.33	4.33	0.82**	23
Purity	91.5	85.0	4. 5**	–
	89.5	86.7	2. 5*	–
Reducing sugar/sucrose ratio (x 100)	2.1	7.3	4. 8**	–
	–	–		–

*+ LSD – *P 0.05, **P 0.01, ***P 0.001*

++ IRSC = (Pol % cane x 0.9) – 1.8

† Data in bold characters are for field sampling technique. Data in light characters are for core sampler.

†† Tons profitable sugar/ha = TC/ha x $\frac{(IRSC - 4)}{100}$

DISCUSSION

The estimation of losses due to yellow spot disease by the use of regular sprays of Benomyl has previously been reported (Egan[6], Ricaud[12]). These have shown that the gains obtained from spraying by these workers and confirmed by the present writers clearly indicated that these are the result of disease control and not a ripening effect of Benomyl. It has been found also that the severe effects of the disease in S 17 are due to its profuse flowering. The lesser response to spraying in vegetative stalks at the "time of harvest may be attributed to the recuperation of such stalks as the disease subsides and new green leaves develop.

Previous attempts to control yellow spot by Benomyl sprays had shown

that their effect did not last long and that 2 sprays applied early, although giving a slight beneficial response, were not efficient (Ricaud[12]). It was observed that 4 to 5 sprays at intervals of 4 to 5 weeks, although not as good as weekly sprays in controlling infection, were nearly as effective in reducing the effects of the disease on sugar content. For many diseases the correlation between loss and amount of infection is not linear (Chiarappa); infection levels must be above a certain threshold for losses to be apparent or important. Present results indicate that this applies for yellow spot. A slight reduction in the infection rate may be sufficient to get returns. It should be pointed out that with the system of disease assessment used, for an infection level of 15%, any of the 4 topmost and efficient leaves has a maximum of 5% spotting.

Although mist blowers could be used for the investigations, their regular use in sugarcane, after the canopy has closed, is excluded. Only some form of aerial application would be practical. In the way the mist blowers were used, however, the lower leaves within the canopy could be treated, and the spray jets hit the under surface of leaves where the fungus sporulates. The topmost leaves were treated at least at their base. The systemic action of Benomyl is translaminar and in the direction of the transpiration stream (Marsh[8]). This ensures a good redistribution of the chemical to the tip of the younger leaves. Although aircraft spraying is used quite successfully in sugarcane for insecticides and ripeners, these are either fully systemic or there is no need for a very good distribution as required for maximum effectiveness with fungicides. The feasibility of aerial spraying of fungicides in a dense canopy crop like sugarcane should not be disregarded. The results of the investigations with overhead spraying, although limited, indicate that Benomyl should be used for aerial spraying against yellow spot. The infection process of this disease has not been studied extensively, but it is generally accepted that infection starts on the younger leaves which are readily infected, and that development on older leaves is due to the spreading of established lesions rather than new infection (Hughes and Ocfemia[7]). Most probably, therefore, only the topmost leaves need to be properly protected against the disease.

However, fungicidal control should not replace the use of resistant varieties. This measure should continue to have priority in the control of yellow spot. Problems which are likely to recur, have cropped up under conditions extremely favorable to the disease, with changes in varietal resistance (Egan[5], Ricaud *et al*[13, 14]). These are possibly due to the selection of strains of the pathogen more virulent to the varieties, or else to assessment of their resistance conducted during periods of low disease intensity. Rapid varietal replacement is not always possible and can be costly, specially with long crop cycles as practiced in Mauritius. Fungicide control can then be a useful temporary measure to reduce losses.

The use of fungicides over wide areas in a field crop is not without problems. The development of resistance to Benomyl has been repeatedly reported in many fungal species (Marsh[8]). To reduce such likelihood, spray alternately with fungi-

cides having different modes of action or apply mixtures of these fungicides.

The economics of spraying are evidently the dominant factor for its adoption. The trials reported, as well as other investigations, indicate potential gains of the order of 2-3° pol % cane, representing 1.3 to 2.0 tons sugar per hectare for cane yield of 75 tons/ha. Discrepancies in estimating gains between the two sampling methods in the industrial trial are explained by the fact that only flowered stalks were taken when sampling in the field. It has also been found in ripener trials that the core sampling technique tends to minimize differences between treatments (R, Julien, private communication). As spraying results in an increase in sugar content, the profitable sugar index should also be considered for comparisons between sprayed and unsprayed plots. Actual returns from spraying will evidently depend on the efficiency of the operation, disease intensity, and cane yield. It is reasonable to expect a potential yield of 1-2 tons sugar per hectare for the variety S17 under severe disease conditions. Four to five sprays would be required, according to climatic conditions. The prospects for economic returns in fungicidal control of yellow spot on variety S 17 in Mauritius are very good and helicopter spraying trials are under way.

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LA FACTIBILIDAD DEL CONTROL CON FUNGICIDAS PARA LA ENFERMEDAD DE LA MANCHA AMARILLA

C. Ricaud, J. C. Autrey y S. Sullivan

RESUMEN

Se evaluó bajo condiciones epifitóticas la eficacia de rociar con benomyl (270 g a.i./ha) para el control de la enfermedad de la mancha amarilla en la variedad susceptible y de florecer profuso S-17. Se compararon las aplicaciones de rociar con una sopladora de neblina a intervalos de 1, 3 y 4 semanas. No hay diferencias entre los tratamientos a intervalos de 3 y 4 semanas. Aunque no se controló la infección tanto como las rociadas semanalmente, éstas fueron casi tan efectivas para darle un aumento sustancial en el contenido de sacarosa. Rociar tambien mejoró la pureza del jugo, pero no hay efecto en el rendimiento de la caña en la cosecha temprano de la temporada. Hay una mejor reacción al control con fungicidas en la caña florecida que en la vegetativa al tiempo de cosecha. En una prueba industrial se obtuvo 57 porciento del control de la infección y un aumento en rendimento de azúcar entre 1 y 2 ton/ha al hacer las aplicaciones de cuatro fungicidas con sopladoras de neblina a intervalos de 5 semanas. El control de la enfermedad fue tambien efectivo cuando se aplicó benomyl sobre el dosel de las hojas con una rociadora de volumen ultra bajo (VUB). Se concluye que el control con fungicidas para la mancha amarilla en la caña de azúcar debe ser factible y económico, sin embargo, no debe reemplazar el uso de las variedades resistentes, pero puede ser una media útil y temporera para reducir las pérdidas en caso de cambiar la resistencia en las variedades hasta que los mismos se reemplacen.

INVESTIGATIONS ON HOST CARRIER RELATIONSHIP OF FIJI DISEASE RESISTANT VARIETIES

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ABSTRACT

Saccharum officinarum L. cultivars resistant to Fiji Disease under test conditions showed symptoms of the disease under high infection doses. It was suspected that the resistant varieties can be symptomless carriers of Fiji disease virus. Such varieties can be a threat to the Fiji disease eradication campaign in Fiji. The results obtained established that the varieties tested do not exhibit the carrier syndrome of Fiji disease causing virus.

INTRODUCTION

In recent years, it was found that some of the new sugarcane varieties released for commercial cultivation have variable disease reactions under field conditions.

The Fiji disease is endemic in *Saccharum* and *Riphidium (Erianthus)* species throughout Fiji and can be transmitted to the commercial crop by the sugarcane leafhopper, *Perkinsiella vitensis* Kirkaldy (Husain and Hutchinson[3]). Thus, Fiji disease will always remain a threat to the Fijian sugar industry. Since there was an assumption that "resistant varieties" can be symptomless carriers of Fiji disease virus, it became an immediate concern that the varieties may pose a continual threat to our efforts to successfully eradicate the disease in Fiji. This study was carried out to assess (i) whether such phenomenon existed in varieties under commercial cultivation, and (ii) whether they were carriers of Fiji disease virus.

MATERIALS AND METHODS

The insectary method of testing varieties for Fiji disease as described by Daniel *et al*[1] was used in the first part of the trial. Twelve single bud cuttings of test varieties were planted in 12 cm x 20 cm polythene bags filled with sterilized soil and supplied with optimum moisture and nutrients for lush growth. The seed was obtained from disease-free seed beds. The test varieties consisted of ten standard varieties and three other commercial clones namely *Kaba, Mali* and *Mana.*

The standard varieties, reaction to Fiji disease (ranging from highly susceptible to highly resistant) was well established from previous trials. These standards were

used in all Fiji disease screening trials. The plants were grown in insectaries so that they were prevented from exposure to viruliferous insects. Six plants of each variety were selected for uniformity of growth. This constituted six replications of each variety. These plants were transferred to another insectary for inoculation.

Nonviruliferous leaf hoppers were collected from sugarcane fields and about 500 insects were caged in Fiji disease-infected Fiji-10 *(Riphidium maximus).* Within five weeks a large number of 3rd, 4th and 5th instar nymphs were available in the cages. These nymphs, averaging about 20 per plant, were 'dumped' in the insectary containing test plants. The nymphs, unable to fly, settled on the leaf sheaths, under the leaf surfaces and the spindle to feed, thus, inoculating the plants with the virus.

After a transmission feed time of 10 days, the insects were killed with household insecticide Insectrol. The plants were removed from the insectary and provided with high moisture and nutrients. After thirty days of inoculation, the plants were examined daily for Fiji disease symptoms. The day the symptoms first appeared was recorded and the diseased plants were discarded.

The varieties were assessed on the basis of the reactions of the first three plants, i.e. half of the replicates. The average of the three replicates was used for the calculation of the resistance ratings.

The SD50 method (Daniels *et al.*[1]) was used to interpret the results. The SD50 is the number of days required for symptoms of disease to appear on 50% of the replicates of a clone. The rating scheme used was the international standardized rating system for recording varietal resistance to sugarcane disease *(Hutchinson*[3]*).* This is a numerical scale raging from 0 (immnune) to 9 (highly susceptible). The ratings are arrived at by first calculating the regression equation using SD50 and the established rating of each of the standard clones; and then calculating the rating of the test variety by using SD50 of the test variety and the regression equation.

The varieties which did not show Fiji disease symptoms after 100 days were separated for seed multiplication. These varieties, namely, *Saccharum officinarum* cultivars *Ragnar, H46-2731, Homer, Kaba, Mali* and *Mana* are highly resistant to Fiji Disease. When enough seed material was available, single-bud cuttings were planted in 20 cm plastic pots filled with sterilized soil. A fresh set of nonviruliferous leaf hoppers were collected from the fields and about 500 insects were caged on the plants of the six varieties mentioned above.

The nymphs were collected from these cages and used for inoculation of the three standard varieties known to be highly susceptible. The three varieties were *Riphidium maximus,* Fiji-10; *S. officinarum,* H52-3157 and *Pindar.* The method described earlier was used. After a 10 day inoculation period, the insects were killed and the plants were removed from the insectaries. The plants were examined daily for Fiji disease symptoms from 30th day onwards.

RESULTS

The results for the first stage of the trial when viruliferous insects were used for inoculation are given in Table 1.

The individual plants of the varieties *Ragnar,* H46-2731, *Homer, Kaba, Mali* and *Mana* which did not show symptoms of Fiji disease were used to assess whether these varieties were symptomless carriers. When leaf hoppers were reared on these plants and the nymps used to inoculate the Fiji disease susceptible *Riphidium maximus,* Fiji-10; *S. officinarum* varieties *Pindar* and H52-3157 no symptoms developed. Varieties Fiji-10; H52-3157 and *Pindar* are highly susceptible to Fiji disease. They normally show symptoms very early in a Fiji disease trial.

TABLE 1. Resistance ratings of varieties to Fiji disease

Varieties	SD 50				Ratings
	Rep 1	Rep 2	Rep 3	Avg.	
Ragnar	100	100	100	100	2
Fiji-10	37	39	42	39	9
H64-2731	100	100	100	100	2
H51-5017	67	69	84	73	5
H52-3157	37	39	45	40	9
H53-3303	60	69	85	71	5
Homer	100	100	100	100	2
Pindar	30	56	60	49	8
POJ-2364	37	52	56	48	8
POJ-3016	42	42	60	48	8
Kaba	100	100	100	100	2
Mali	100	100	100	100	2
Mana	100	100	100	100	2

DISCUSSIONS

The replicated experiments indicated that the six resistant varieties under investigation did not act as carriers of Fiji disease virus even though the plants were exposed to a large number of viruliferous leaf hoppers and therefore to heavy doses of virus.

It seems unlikely that *S. officinarum* cultivars can be classes as symptomless carriers of Fiji disease virus. The previous assumption is therefore invalid. However, the fate of the virus is unknown. It may be that the resistance process in the plant eliminates the virus thus making the plants free of the virus introduced by the viruliferous hoppers.

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INVESTIGACIONES SOBRE LAS RELACIONES DE HUESPED PORTADOR EN VARIEDADES RESISTENTES A LA ENFERMEDAD DE FIJI

K. Reddy

RESUMEN

Cultivares resistentes de caña *(Saccharum officinarum)* a la enfermedad de Fiji mostraron sintomas de la enfermedad bajo condiciones de prueba bajo alta dosis de infeccion. Se sospechaba que las variedades resistentes pueden ser portadoras asintomaticas de la enfermedad viral de Fiji. Tales variedades pueden ser una amenaza para la campaña de erradicacion de la enfermedad de Fiji en Fiji. Se llevaron a cabo investigaciones para determinar (i) si dicho fenomeno existia en variedades bajo cultivo comercial y (ii) si eran portadores de la enfermedad. Se ha establecido que las variedades probades no exhiben el sindrome del portador del virus de la enfermedad de Fiji.

FACTORS THAT INTERFERE IN EVALUATION OF RATOON STUNTING DISEASE RESISTANCE BY WATER FLOW IN SUGARCANE STALKS I

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ABSTRACT

Ratoon Stunting Disease (RSD) control in sugarcane has not been satisfactory mainly because of the difficulties in its identification and application of thermotherapy. The development of cultivars resistant or tolerant to RSD is difficult because on rapid and efficient screening method is available.

As an attempt to solve the problem the water flow method was developed to measure RSD resistance in sugarcane cultivars. The results indicated that factors such as vacuum pressure, position of node in sugarcane stalks and days of storage interfered in RSD resistance evaluation by the water flow method.

INTRODUCTION

Of the diseases that attack sugarcane in Brazil, the ratoon stunting disease (RSD) is considered the most important because it can be disseminated after a year without being detected and the hot water treatment is difficult to apply for several reasons.

In some cultivars, disease identification in the field can be done based upon symptoms of mature stalks. The accuracy of this identification depends upon cultivars, stalk age and absence of other vascular diseases that can cause symptom similar to RSD.

Until 1973 the ratoon stunting disease was considered as caused by virus, Schexynayder[14]; Forbes and Ling[7]; Steindl[15]; Carvalho[4]; Gillaspie[8]. After this year, the evidence that bacteria were always associated to the vascular discoloration symptom in RSD was reported by several authors. Bailey[3], Gillaspie *et al*[9]; Ricaud *et al*[12]; Worley and Gillaspie[18]; Tokeshi *et al*[17].

The use of cultivars resistant to RSD has not been practiced efficiently since no breeding program has been directed specially for this purpose obtaining RSD tolerant cultivars depends on chance.

In several vascular diseases it was found that vascular anatomy was involved in resistance mechanism, (Peltier and Schroeder[12], Jones[10], Cho *et al*[6], Saalting and Dimond[13], Elgersma[6], Atkinson[2], and Abbott and Hughes[1]). In sugarcane, Teakle *et al*[16] investigated the hypothesis that vascular anatomy was involved in resistance to RSD. They measured water flow in RSD resistant and susceptible cultivars, studied the number and anatomy of vascular bundle of these plants and concluded that the number of xylem vessel passing continuously through the node was larger in RSD susceptible cultivars.

This research was conducted as an attempt to evaluate factors that might interfere in the RSD resistance evaluation through the water flow method in diseased and healthy stalks. The research was carried out in the Department of Plant Pathology, College of Agriculture, ESALQ, University of Sao Paulo.

The facilities and material were furnished by PLANALSUCAR Experimental Station at Araras, S.P. and COPERSUCAR, Esp. Station at Piracicaba.

MATERIALS AND METHOD

The method used in this research was the same as that applied by Valarini and Tokeshi. Water was passed through healthy and diseased stalks of cultivars susceptible to RSD using the equipment shown in Figs. 1 and 2. The water flow from stalks, two RSD indicator varieties IAC 52/325 and CP42-184, was determined under controlled conditions in provet placed inside of Kitasato's vacuum flask for 15 minutes.

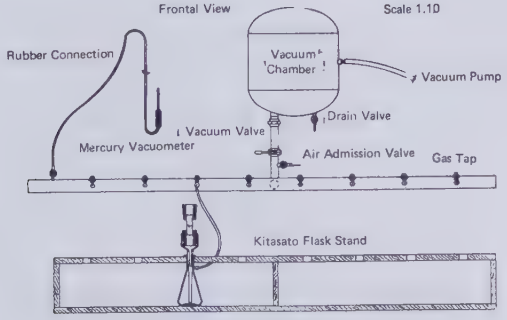

FIGURE 1. Equipment used in water flow measurement

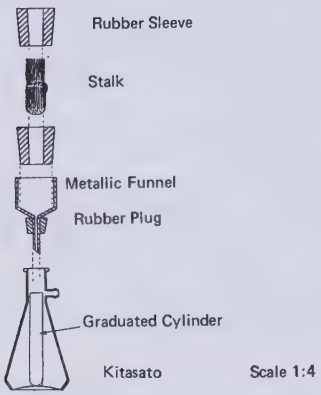

FIGURE 2. Apparatus used to connect stalks to the vacuum system

While the water flow was being determined, the following factors were studied: vacuum pressure from 30 to 70 mm of Hg; the effect of basal, middle and, apical node position and storage from 0 to 1 day in water flow of healthy and diseased stalks growing in identical conditions during the same period.

RESULTS AND DISCUSSION

The effect of vacuum pressure in water flow (Table 1), indicated that increasing the negative pressure caused a higher water flow, and caused more problem to keep the air tight system. Since the water flow difference between healthy and diseased stalks was great in all studied vacuum conditions, the negative pressure of 30 mm Hg was applied in all tests for practical reason.

TABLE 1. Effect of vacuum pressure in mean water flow of sugarcane stalks of healthy and diseased (RSD) plants

Vacuum in mm of Hg	Mean flow of 20 stalks per treatment in cm^3 in 100 min.			
	Cultivars			
	IAC 52/326		CP45-184	
	Diseased	Healthy	Diseased	Healthy
30	15.86	54.12	28.62	110.34
50	18.70	36.75	*	*
70	224.50	293.50	152.50	256.69

**plot lost by accident*

These results are comparable to those obtained by Teakle *et al*[16] and probably will cause less mechanical damage to the vascular system of the stalks.

The node position on stalks also can cause changes in water flow. Table 2 shows that the water flow between healthy and diseased plants was statistically different at 1% level of probability using the "t" test in all tested positions. On the other hand, within the same treatment, healthy or diseased, comparison between position AB, AC or BC were statistically similar using the Tukey test. The results indicated a tendency to increase the water flow from basal node toward apical node. The coefficient of variation indicated that some uncontrolled factors were interfering in the results. The difference in water flow between healthy and diseased stalks may be attributed to disease distribution inside the stalks age maturation and different cultivars.

TABLE 2. Effect of node position in healthy and diseased (RSD) stalks of cultivar CP45-184 on mean water flow

Number of stools with 6 to 10 stalks	Mean flow of node in different position in cm^3 in 100 minutes and vacuum pressure of 30 mm Hg					
	Diseased			Healthy		
	Node position*			Node position		
	A	B	C	A	B	C
1	22.09	27.09	40.01	128.72	150.08	130.72
2	42.52	47.52	75.03	112.27	136.74	141.18
3	21.26	30.01	40.85	90.04	135.07	130.90
Mean**	28.62a	34.87a	51.96a	111.72b	140.63b	134.26b
C.V.	62.50	50.90	49.95	38.82	33.34	41.73

**Node position starting from ground level A = node 4 or 5; B = node number 8 or 9; and C node number 12 or 13*

***Within each cultivar, means with the same letters are statistically similar*

The results were obtained from the basal node (position A only for all treatments because lower frequency of stalk borer damage by *Diatraea saccharalis* were observed.

From the data shown in Table 3, it is evident that 24 hours storage

TABLE 3. Effect of storage period in mean water flow in healthy and diseased (RSD) stalks plants

Determination Period	Mean* flow of 20 stalks per treatment in cm^3 in 100 minutes at vacuum pressure of 30 mm Hg			
	Cultivars			
	IAC 52/326		CP45-184	
	Diseased	Healthy	Diseased	Healthy
After harvesting	15.97a	54.54b	28.62a	111.72b
C.V.	63.83	55.56	62.50	38.82
24 hours after Harvesting	14.49a	26.52b	5.93a	13.52b
C.V.	88.00	91.34	65.37	87.33

**Within each cultivar means with the same letters are statistically similar*

caused lower water flow. The decrease was more conspicuous in healthy than in diseased treatment. The difference in mean flow between healthy and diseased measured after havresting or 24 hours later was statistically significant. But the difference of flow after one day of storage demonstrated that longer period of storage is not recommended. The smaller coefficient of variation obtained from the data just after harvesting also indicated that the flow evaluation should be done as early as possible to get more reliable results and that it was necessary to use larger sample size.

CONCLUSION

Based on the water flow measurement obtained from healthy and diseased stalks, vacuum pressure, position of node in stalks and storage period affects RSD resistance evaluation by the water flow method and that it is always necessary to set and compare each variety under the same identical conditions.

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FACTORES QUE AFECTAN LA EVALUACION DE LA ENFERMEDAD DE ENANISMO RETOÑO DE LA CAÑA DE AZUCAR POR EL METODO DEL FLUJO DE AGUA EN LOS TALLOS DE LA CAÑA DE AZUCAR

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RESUMEN

El control de la enfermedad de enanismo de retoño (EER), no ha sido satisfactorio principalmente por las dificultades de su identificacion y aplicacion de termoterapia. El desarrollo de variedades resistentes o tolerantes a EER es dificil porque no hay metodo de evaluacion rapido y eficiente disponible.

Para resolver el problema, se desarrollo el metodo del flujo de agua para evaluar la resistencia de las variedades de la caña de azucar a EER. En esta investigacion como un complemento al metodo del flujo de agua, se determino la interferencia de varios factores.

Los resultados indicaron que factores tales como presion de vacio, posicion de nudo en los tallos de la caña de azucar y dias de almacenamiento interfieren con la evaluacion de la resistencia a EER por el metodo del flujo de agua.

MICROPLOTS FOR SCREENING SUGARCANE VARIETIES FOR TOLERANCE TO RATOON STUNTING DISEASE

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ABSTRACT

Tolerance of sugarcane varieties to ratoon stunting disease (RSD) has been evaluated in small field plots (microplots) comprising four plants 50 cm apart and in 1.50 m inter-row distance. Two trials were carried out, in a split-plot design, using progeny of heat treated cuttings as healthy plants and progeny of RSD-inoculated as diseased plants. Those treatments constituted the sub-units. Three check varieties were included as common varieties for both trials. Weight of stalks in the microplots were obtained from plant cane and first ratoon.

The three check varieties were also tested, together with a fourth variety, in a comparative trial, planted according to the current commercial system in normal size plots. The results obtained for these varieties in the microplot trials, showed a close agreement to those of the comparative trial. This fact indicates the validity of the microplot system for screening RSD varietal tolerance.

It is suggested that further studies to test each variety in at least two distinct location/soil type, increasing a bit the number of plants of each sub-unit and also doubling the inter-row and inter-plot distance, in order to avoid the deleterious shading effects.

The method could be valuable for screening RSD tolerance of large number of varieties, with a minimum of labor, land and operating costs.

INTRODUCTION

Ratoon stunting disease (RSD) is considered to be causing economic losses, throughout the sugarcane growing areas of the world, bigger than any other disease of this crop, (Hughes[6], Steindl[14]).

After its first record in Queensland in 1944-45 and the arduous studies pursued by Steindl and Hughes[16], it has been established that the control of this systemic disease can be achieved by seedpiece heat treatment.

In commercial conditions, however, it has been observed that a complete cure is not attained by any heat treatment technique, and retreatment over the years in a regular basis is necessary to keep the disease at a minimum (Steindl[15]). Nevertheless, it has rarely been considered that the net profit of this practice is strongly dependent on the degree of intolerance of the variety to the disease. Cases do occur when heat treatment is not gaining, due to some tolerance of the variety. The knowledge of this tolerance level is, consequently, an important tool for rational control recommendations.

The critical determination of RSD tolerance of sugarcane varieties is a difficult task since the only way is measuring the effect on yield, which is greatly influenced by extra-factors like soil type, soil fertility (Wang[20]) and especially soil moisture content (Rossler[13], Steindl[14] and Wang[20]). Owing to these, yield losses as a result of RSD infection may vary greatly from trial to trial, in some cases with no significant losses in a specific susceptible variety. Consequently, data on varietal tolerance based on routine experimental designs cannot be critically compared between experiments carried out at different times and/or localities.

Another difficulty is that routine experimental plots adopted in sugarcane research requires considerable operating costs, labor and physical facilities, making it impracticable when the number of varieties to be studied comprises a dozen or more.

In trying to solve these difficulties, experiments with microplots have been established in an attempt to develop a relatively simple and rapid screening method for a large number of varieties, combined with statistical requirements for analysis of distinct experiments.

MATERIALS AND METHODS

Nurseries of healthy and diseased plants of all test varieties were prepared a year ahead. Three-eyed cuttings were hot-water treated at 50°C for 2 hours and planted in the field to establish the RSD-free nursery. The diseased nursery was planted with three-eyed cuttings immersed for 5 minutes in juice extracted from RSD-infected stalks diluted 1:5 with water.

In 1976, two microplot tests were installed in a dark-red ortho latosolic-soil at the Southern Central Experiment Station of PLANALSUCAR, Araras - SP (latitude 22° 18' S). A split-plot design, with four replications was adopted. Diseased and healthy sub-plots of each variety were randomly paired. In March and April 76, one-eyed cuttings were obtained from the above mentioned nurseries and germinated in small plastic bags filled with soil. Later, the plants were space-planted to the field 0.50 m apart. Each sub-plot of one row comprised

4 plants, with a gap of one meter separating sub-plots of the same row. The distance between rows was 1.50 m. All the blocks of the tests were displayed in 8 rows with East-West orientation. Sprinkler irrigation was applied as needed till complete establishments of the plants.

Trial I, comprising 17 varieties, was set on May 11, 1976, in 600 m^2 plot, Trial II with 21 varieties planted on June 3, 1976 in 360 m^2 plots. Three varieties were common in both trials.

TABLE 1. Mean effect of ratoon stunting disease on the yield of 17 varieties in microplot test. Plant cane, first ratoon and combined data. (Trial 1).

Variety	Crop	Cane yield (kg/plot) Healthy	Diseased	Diff.
	Pc	57.07	52.62	− 4.45 ns
CB36-24	R	38.30	19.23	−19.07 **
	a	47.69	35.93	−11.96 *
	Pc	75.07	58.42	−16.65 *
CB40-69	R	55.90	24.78	−31.12 **
	a	65.49	41.60	−23.89 **
	Pc	73.10	62.05	−11.05 ns
CB41-14	R	50.53	28.30	−22.23 **
	a	61.82	45.18	−16.64 **
	Pc	83.20	63.10	−20.10 **
CB41-76[c]	R	48.38	20.40	−27.98 **
	a	65.79	41.75	−24.04 **
	Pc	45.90	37.12	− 8.78 ns
CB46-47	R	28.83	19.30	− 9.53 ns
	a	37.37	28.21	− 9.16 ns
	Pc	54.80	58.42	+ 3.62 ns
CB47-355	R	39.63	36.45	− 3.18 ns
	a	47.22	47.44	+ 0.22 ns
	Pc	69.45	44.23	−25.22 **
CB50-34	R	43.85	12.98	−30.78 **
	a	56.65	28.61	−28.04 **
	Pc	54.80	48.57	− 6.23 ns
CB60-31	R	31.43	23.55	− 7.88 ns
	a	43.12	36.06	− 7.06 ns
	Pc	60.30	56.57	− 3.73 ns
CP51-22	R	59.26	32.53	−26.73 **
	a	59.78	44.55	−15.23 *
	Pc	64.65	46.10	−18.55 *
CP52-48	R	44.43	21.75	−22.68 **
	a	54.54	33.93	−20.61 **

	Pc	59.28	57.78	– 1.50 ns
IAC47-31	R	34.58	25.08	– 9.50 ns
	a	46.93	41.43	– 5.50 ns
	Pc	54.15	45.32	– 8.83 ns
IAC49-131	R	54.95	22.68	–32.27 **
	a	54.55	34.00	–20.55 **
	Pc	68.62	59.62	– 9.00 ns
IAC50-134	R	50.75	36.03	–14.72 *
	a	59.69	47.83	–11.86 *
	Pc	67.57	55.82	–11.75 ns
IAC52-326[c]	R	48.08	17.58	–30.50 **
	a	57.83	36.70	–21.13 **
	Pc	62.62	54.65	– 7.97 ns
IAC55-26	R	42.08	27.00	–15.08 *
	a	52.35	40.83	–11.52 ns
	Pc	69.30	62.75	– 6.55 ns
NA56-79[c]	R	44.03	35.15	– 8.88 ns
	a	56.67	48.95	– 7.72 ns
	Pc	41.97	33.10	– 8.87 ns
PR1085	R	21.25	5.85	–15.40 *
	a	31.61	19.48	–12.13 *

Pc = plant cane; R = first ratoon; a = average of Pc and R.
c = Varieties common for trials I and II.
ns = non significant according to Tukey test;
** = significant at 5% level;*
*** = significant at 1% level.*

A third trial was set on May 28, 1976 between Trial I and II in the same 8 rows. Three-eyed cuttings of the diseased and RSD-free nurseries of four varieties were planted in a split design. Each subplot comprised two rows of 6.7 m. The planting was done in the commercial manner, in a rate of 4 cuttings/m. This test aimed at comparing the results of microplot experiments with commercial planting system.

Plant cane was harvested in October, 1977, taking care to avoid contamination of healthy plots by the knives. The same care was taken in the first ratoon crop, harvested from November through December, 1978. The blocks were harvested on different days to make possible the use of the stalks in a study of water flux through the vessels of healthy and RSD-infected canes (Valarini[19]).

TABLE 2. Mean effect of ratoon stunting disease on the yield of 12 varieties in microplot test. Plant cane, first ratoon and combined data (Trial II).

Variety	Crop	Cane yield (kg/plot) Healthy	Diseased	Diff.
	Pc	67.17	52.42	−14.75 *
CB41-76[c]	R	42.18	23.78	−18.40 **
	a	54.68	38.10	−16.58 **
	Pc	78.47	73.37	− 5.10 ns
CB45-3	R	59.30	42.50	−16.80 **
	a	68.89	57.94	−10.95 *
	Pc	70.20	54.17	−16.03 **
CB45-155	R	49.53	31.15	−18.38 **
	a	59.87	42.66	−17.21 **
	Pc	57.50	52.15	− 5.35 ns
CB49-62	R	38.25	34.75	− 3.50 ns
	a	47.88	43.45	− 4.43 ns
	Pc	48.12	37.57	−10.55 ns
CB56-20	R	32.15	20.13	−12.02 ns
	a	40.14	28.85	−11.29 *
	Pc	64.62	44.58	−20.04 **
CB53-98	R	46.60	22.45	−24.15 **
	a	55.61	33.52	−22.09 **
	Pc	47.82	41.87	− 5.95 ns
Co775	R	29.95	15.27	−14.68 *
	a	38.89	28.57	−10.32 *
	Pc	40.02	25.35	−14.67 *
CP53-17	R	22.35	5.97	−16.38 *
	a	31.19	15.66	−15.53 **
	Pc	67.32	59.07	− 8.25 ns
IAC51-201	R	57.78	34.70	−23.08 **
	a	62.55	46.89	−15.66 **
	Pc	67.22	56.30	−10.92 ns
IAC51-205	R	45.30	39.25	− 6.05 ns
	a	56.26	47.78	− 8.48 ns
	Pc	65.10	51.70	−13.40 *
IAC52-326[c]	R	31.50	13.70	−17.80 **
	a	48.30	32.70	−15.60 **
	Pc	54.52	45.55	− 8.97 ns
NA56-79[c]	R	35.00	27.08	− 7.92 ns
	a	44.76	36.32	− 8.44 ns

Pc = plant cane; R = first ratoon; a = average of Pc and R.
c = Varieties common for trials I and II.
ns = non significant according to Tukey test;
** = significant at 5% level;*
*** = significant at 1% level.*

TABLE 3. Mean effect of ratoon stunting disease on the yield, of 4 varieties in competitive plots. Plant cane, first ratoon and combined data. Trial III.

Variety	Crop	Cane yield (ton/ha) Healthy	Diseased	Diff.
CB41-14	Pc	153.0	128.1	−24.9 ns
	R	106.3	60.9	−45.4 **
	a	129.7	94.5	−35.2 *
CB41-76	Pc	148.6	112.2	−36.4 *
	R	93.8	60.3	−33.0 *
	a	120.9	86.2	−34.7 *
IAC52-326	Pc	149.3	97.8	−51.5 **
	R	97.6	26.1	−71.5 **
	a	123.4	61.9	−61.5 **
NA56-79	Pc	108.1	120.5	+12.4 ns
	R	88.3	73.4	−14.9 ns
	a	98.2	96.9	− 1.3 ns

CV(a) = 8.54% (Pc); 18.13% (R) and 11.74% ($\bar{a}$)
CV(b) = 15.33% (Pc); 25.15% (R) and 18.73% ($\bar{a}$)

Pc = plant cane; R = first ratoon; a = average of Pc and R.
ns = non significant according to Tukey test;
* = *significant at 5% level;*
** = *significant at 1% level.*

RESULTS

A very high difference in yield between healthy and diseased plots was obtained in both crops and in the combined data.

Results in yield per plot of plant cane, first ratoon and combination of both in Trials I, II and III are presented in Tables 1, 2 and 3, respectively. As can be seen, the common varieties CB41-76, IAC52-326 and NA56-79 performed quite similarly in the three trials, i.e., in the two microplot trials and in the competitive trial.

Another variety, CB41-14, also performed similarly in the microplot trial (Table 1) and the competitive trial (Table 3).

From the practical standpoint, in Trial I, varieties IAC40-69, CB41-14, CB41-76, CB50-34, CP52-48, IAC49-131 and IAC52-326 performed as highly susceptible; varieties CB63-24, CP51-22, IAC50-134, IAC55-26 and PR1085 as moderately susceptible; CB46-47, CB60-31, IAC47-31 and NA56-79 as tolerant; and CB47-355 as highly tolerant.

In Trial II, varieties CB41-76, CB45-155, CB53-98, CP53-17, IAC51-201 and IAC52-326 performed as highly susceptible CB45-3, CB56-20 and CO775 as moderately susceptible; and CB49-62, IAC51-205 and NA56-79 as tolerant.

The criteria adopted for this classification are as follows:

Highly susceptible varieties are those with yield reduction significant at 1% level at least in first ratoon and in the combined data; moderately susceptible varieties with yield reduction not significant in plant cane and significant at 5% level in the first ratoon and/or in the combined data; tolerant varieties with some yield reduction but not statistically significant; and highly tolerant variety without yield reduction.

DISCUSSIONS

The only method presently known to assess varietal tolerance to RSD is by determination of yield difference between healthy and diseased plots, although water flux through vessels is being studied as a possible tool (Teakle *et al*[17], Valarini[19]).

In Queensland, commercial varieties have been routinely tested against RSD by comparison of paired plots of healthy and diseased canes. (Hughes and Steindl[7]). However, this empirical method has been substituted by another that make possible statistical analyses (BSES[3]).

In Louisiana, USA, they had as routine testing of new varieties small field plot tests with 2 control varieties and 4 or 5 replications. The yield performance is extended through the first stubble crop and 6 to 8 new varieties are tested each year (Abbott and Zummo[1]).

Whichever method was employed, the considerable operating costs, labor and land requirements are evident. This is more true in breeding programs like those in Brazil, where a large number of varieties comes to the scene every year, besides dozens of the old commercial varieties.

In trying to solve these difficulties a study with microplots has been initiated, as has been suggested for studies with cereal crops (Briggs and Shebeski[2], Frey[5], Jensen and Robson[8]). Moreover, small plot was utilized in the early stages of selection of sugarcane breeding programs. Wismer[21] tested 34 Hawaiian varieties against RSD in single plant paired plots, with 20 replications.

Although originally it had been planted to install the two tests in distinct locations and/or soil types, operating problems led to set them up almost in the same conditions. The only difference was the one month lapse of planting between both.

It is well known that losses by RSD in a specific variety is influenced by soil and environmental effects. Even in varietal performance trials, the relative performance of the same varieties changes considerably when plantings are made in different years (BSES[4]). Results of several experiments in Brazil have shown that varieties CB41-76 and IAC52-326 are very sensitive to RSD (Matsuoka[11], Matsuoka and Aguiller[12]). In all of them, CB41-76 showed significant losses even in plant cane, as it did in the three trials reported here. Variety IAC52-326, however, only showed significant losses in first and second ratoons of two experiments carried out at the Araras Southern Central Experiment Station, from 1975 to 1978 (Matsuoka, unpublished; Dodson *et al*, unpublished). On the other hand, this variety showed significant yield reduction even in plant cane in trial III, as it did in another experiment carried out at Lencois Paulista-SP in a dark red distrofic latosolic soil (Matsuoka and Aguillera[12]).

The variety NA56-76 showed no statistical difference in both microplots trials and also in the competitive trial, neither in plant cane nor in first ratoon. However, this variety showed high yield difference between healthy and RSD-diseased plots in a test carried out at Lencois Paulista-SP (Matsuoka and Aguillera[12]).

All these figures clearly indicate that the RSD effect is strongly influenced by extra-factors and so, at least two experiments in different years or locations have to be set up for reliable results. This point shows why a small plot technique could be valuable for determination of varietal tolerance to RSD using yield as parameter, as the problem of land and labor would be more critical.

Spaced planting system could be criticized because of the poorly understood relationship between variety performance in spaced plots and in high-density commercial type plantings. However, Lyrene[9] stated that it is apparent that some genotypes are inherently weak and do poorly in most situations while others are inherently vigorous and perform well under a wide range of conditions. In addition, Lyrene and co-workers[10] concluded that there was a positive correlation between mean stalk number per spaced-stool and mean stalk number per competitive plot.

As the test proposed aims at detection of the potential tolerance of the variety to RSD and diseased and healthy plots of the same variety are both grown in the same spaced conditions, this technique probably offers no error. Besides, it permits uniform stand all over the experiment, as replanting can be made wherever necessary. Nevertheless, the deleterious shading effect in these small plots needs strong consideration (Tovey *et al*[18]). As healthy and RSD-diseased plants show markedly height differences, no doubt exists that the diseased plots were excessively unfavored by the decrease in

the amount of radiation received. Consequently, the damage of RSD in a specific variety can be overemphasized. For instance, it was observed in the first ratoon of PR1085 in Trial I, a very clear canopy height difference from other varieties of the test, even bigger in the diseased plots. Much of this stunting effect can be attributed to the deleterious effect of shading. Moreover, it was observed that if the plants are not carefully chosen before transplanting to the field, some defective plants could die or be excessively stunted by shading of more vigorous neighbor plants. The high coefficient of variation of the tests here reported could be in part attributed to this undesirable effect.

The deleterious shading effect probably can be minimized by an alternative larger inter-row and inter-plot spacing. Increasing the number of plants per plot to six, doubling the inter-row spacing to 3.0 m and the gap between sub-plots of the same row to 1.5 m, probably will diminish shading to an acceptable level without losing, the microplot characteristics, though this proposal will need further studies. Also, it must be considered if the orientation of the rows (Tovey *et al*[18]) will significantly affect shading even in this new plot structure.

In breeding programs where yearly a number of varieties is liberated for commercial plantings, it is worthwhile to previously know their tolerance to RSD. In this case, the advantage of a microplot technique can be visualized. The number of varieties to be tested can be divided in smaller groups and each of them tested in distinct locations and/or years; also, different year-bred varieties can be independently tested, always providing the same check varieties in the tests. Later on, statistical comparisons can be made and relative tolerance of varieties can be quite critically established.

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MICROPARCELAS PARA SELECCIONAR VARIEDADES DE LA CAÑA DE AZUCAR CON TOLERANCIA A LA ENFERMEDAD DEL ENANISMO DEL RETOÑO

S. Matsuoka

RESUMEN

Se ha evaluado en las parcelas pequeñas (microparcelas) la tolerancia de las variedades de la caña de azucar al enanismo del retoño. Estas consisten de 4 plantas a una distancia de 50 cm entre plantas y 1.50 m entre hileras. Se hicieron dos pruebas utilizando un diseño de parcelas divididas (split plot) con proles de las semillas tratadas con agua caliente como sana y las inoculadas por RSD como enfermas. Se incluyeron tres variedades de testigo como variedades comunes para ambas pruebas. Se obtuvieron los pesos de la caña en las microparcelas en plantilla y en primer retoño.

Las tres variedades de testigo fueron probadas tambien junto con una cuarta variedad en una prueba comparativa, sembrado segun el sistema comercial actual en parcelas con tamaño normal. Los resultados obtenidos para estas variedades en las pruebas con microparcelas indican que estan completamente de acuerdo con los de la prueba comparativa. Se sugirió la validez del sistema de microparcelas para selecionar la tolerancia de las variedades a RSD.

WALLABY EAR DISEASE IN SUGAR CANE IN QUEENSLAND

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ABSTRACT

A disease which caused gall formation on the abaxial surface of sugarcane leaves and in some cases, severe stunting of stools, was located in several canegrowing districts of Australia in 1976. Similar symptoms were also observed in several grasses growing adjacent to affected cane fields.

Investigations showed that *Cicadulina bimaculata*, after feeding on diseased sugarcane, could produce symptoms in maize assay plants, which were similar to those caused by the maize wallaby ear virus (MWEV). Spherical particles, similar in size to those of MWEV (c. 45 nm), were seen in extracts from infected maize assay plants but not in extracts from infected sugarcane.

This is the first record of wallaby ear disease naturally occurring in sugarcane in the field. The structure of galls on sugarcane caused by MWEV and Fiji disease were compared. Galls on sugarcane affected with MWEV were apparently formed mainly by proliferation of parenchyma cells.

INTRODUCTION

In late November, 1976 severely stunted stools were observed in a young crop of the sugarcane variety Q90 in the Mulgrave mill area of north Queensland, Australia. Examination of these stunted stools and adjacent cane revealed the presence of galls on the abaxial surface of leaves. A large number of the leafhopper *Cicadulina bimaculata* (Evans) was also present in the cane.

The symptoms in affected cane were initially confused by field workers with those produced by Fiji disease, a disease which causes serious economic losses in south Queensland (Anon[1]). Investigations were initiated to determine the cause of the stunting and gall formation in Q90. The symptoms and presence of *C. bimaculata* suggested that the disease may be caused by the maize wallaby ear virus (MWEV), although the symptoms differed from those reported by Grylls[2] who had

observed the effect of MWEV on various Gramineae in a glasshouse study symptoms in sugarcane were the production of an excessive number of distorted tillers but with no galls present.

Wallaby ear disease occurs in a number of maize cultivars grown in Queensland. The name was derived from the similarity of stiff, erect leaves of disease plants to the erect ears of the wallaby (Grylls[2]).

This paper presents evidence to show that MWEV was the cause of the disease in Q90 in the Mulgrave area. It also reports on the symptoms of the disease, structure of galls, incidence in sugarcane in Queensland, and some possible alternative hosts of the virus.

SYMPTOMS OF THE DISEASE

The disease can be recognized by the presence of small galls 1 to 2 mm in length, 1 to 1.5 mm in width, and up to 1mm in height on the lower surface of leaf blades of affected cane. These galls differ from those of Fiji disease in that they have clear vein down the center (Fig. 1) while the Fiji disease galls do not have (Fig. 2). When the galls are numerous, they produce a mottled effect on the upper surface of leaves, particularly towards the base of the leaf blade.

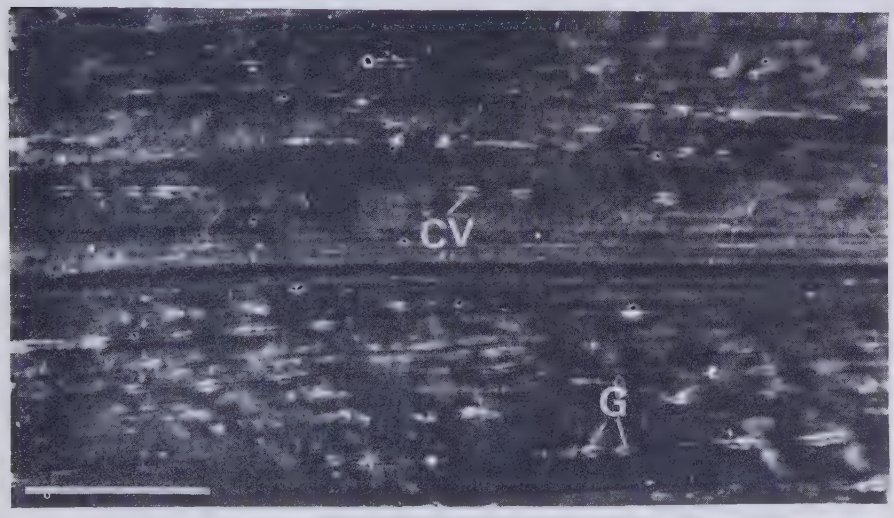

FIGURE 1. Gall formation (G) under surface of leaf affected by wallaby ear disease. A clear vein (CV) passes through the center of each gall. (Bar represents 10 mm)

The disease may cause severe stunting of stools with the most affected having short, stiff and erect leaves similar to those of the Fiji disease (Fig. 3). There appears to be recovery from the disease and mature cane in the original, severely affected field showed little evidence of the disease in mid-1977 except that some stalks were thinner than normal.

MATERIALS AND METHODS

Transmission studies

Three-month old stools of diseased Q90 plant cane were collected from the field and planted in pots in the insectary of the Entomology Division of CSIRO, Canberra.

The insects used to assay for MWEV in sugarcane were virus-free *C. bimaculata* from a colony that had been established during research on wallaby ear disease of maize (Grylls[2]). It had been maintained in an insectary on healthy maize seedlings of the cultivar L. 12.

Virus-free insects were given a five to six day acquisition feeding on diseased sugarcane plants, and were then caged (five/plant) on 12 seedlings of L. 12, a maize cultivar susceptible to infection with MWEV. After five days inoculation access time, the insects were removed and the maize seedlings were held for observation.

Virus-free leafhoppers were later caged on those maize test seedlings which had developed leaf gall symptoms. These insects in turn were caged (five/plant) on 16, three-week old disease-free plants of Q90 for inoculation feeds.

Virus purification and electron microscopy

Leaves from infected maize plants from the initial transmission experiment, and leaves from infected sugarcane collected from the field were processed separately. Extraction and purification procedures were similar to those described by Grylls[2]. A drop of each preparation was placed on a carbon coated formvar grid, stained with two percent sodium phosphotungstate, and examined in a Joel electron microscope.

Anatomy of galls

Free-hand sections from leaf tissues of Q90 stained in two per cent acqueous safranin were examined microscopically and compared with sections from galls produced by Fiji disease on the variety POJ2714. These latter sections had been stained with iron haematoxylin and bismarck brown as described by Johansen[4]

Incidence of the disease

Young plant cane in north Queensland was surveyed for the presence of the disease. The most intense surveys were made in the Mulgrave and Babinda mill areas.

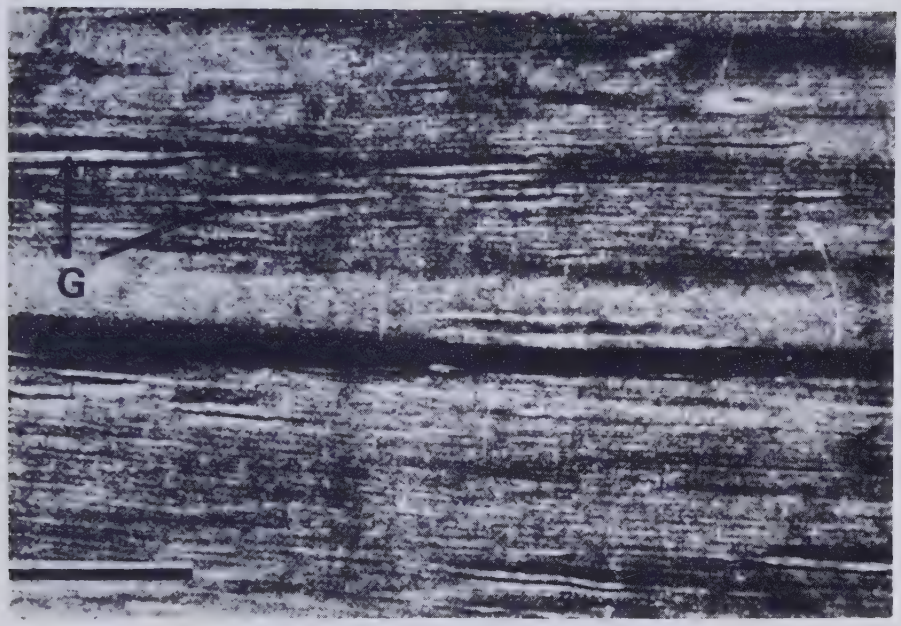

FIGURE 2. Gall formation (G) under surface of leaf affected by Fiji disease. (Bar represents 10 mm)

FIGURE 3. Stools of young Q90 severely infected with MWEV in the Mulgrave district, Queensland

RESULTS

Transmission studies

Twelve maize seedlings were inoculated by *C. bimaculata* which had been given an acquisition feed on diseased cane. Ten of these seedlings developed galls similar to those caused by MWEV. Symptoms appeared 10 to 12 days after the beginning of the inoculation feed.

Galls also developed on 10 of the 16 sugarcane plants which had been fed on by leafhoppers which acquired virus form the maize assay plants. Symptoms appeared on the sugarcane plants 17 to 21 days after the inoculation feed, but they were not as distinct as those on the field-infected cane. Nymphs of *C. bimaculata* later developed from eggs laid in cane during the inoculation access time, and adults arising from these caused severe wallaby ear symptoms on maize seedlings on which they fed.

Virus purification and electron microscopy

Grids prepared with extracts from maize assay plants showed isometric virus particles c. 45 nm in size, and these appeared to be identical with those of MWEV observed by Grylls[2] (Fig. 6). No particles were seen in extracts from infected sugarcane leaves.

Examination of sections of the galls on Q90 revealed that they are apparently formed by proliferation of parenchyma cells plus some bundle sheath cells (Figure 4). However, a detailed investigation is needed to confirm this observation. These galls differ from those caused by Fiji disease, which are formed by proliferation of phloem and xylem cells (Hatta and Francki[3]) (Fig. 5).

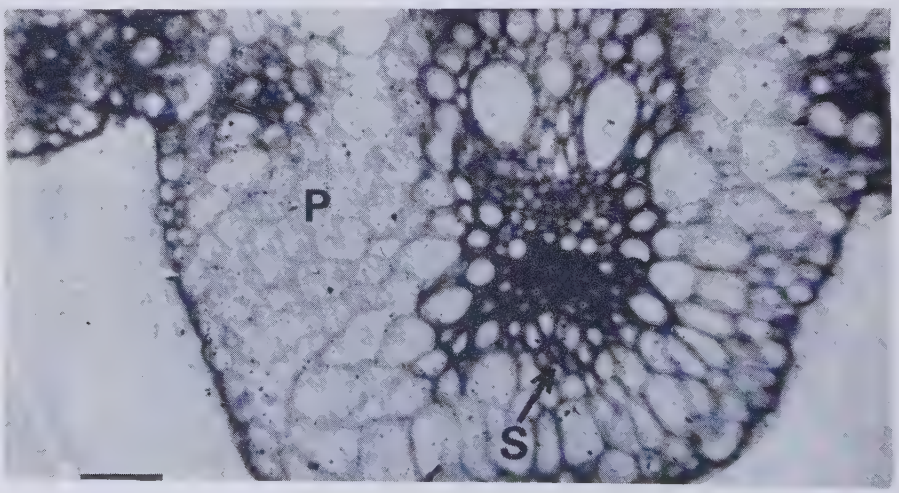

FIGURE 4. Cross section of a gall of wallaby ear disease showing proliferation of parenchyma (P) and sheath cells (S). (Bar represents 100 μ).

Although the disease was located on 18 and 24 farms in the adjoining Babinda and Mulgrave mill areas, respectively, it could not be found in several other mill areas nearby. Most of the infection was in Q90, the major variety grown in the area. In addition, the disease was also found in Q77, Q99, Q101 and Q105. The disease was also observed in cane in southern Queensland at the Bundaberg Sugar Experiment Station and the BSES Pathology Farm, Brisbane.

The heaviest infection was in the block of Q90 in the Mulgrave area in which the disease was first observed. This may have been due to the very large number of leafhoppers present. The cause of this infestation is unknown.

Subsequent ratoons in all disease blocks appeared to be healthy.

Alternative hosts

During inspections of infected cane fields the following grasses were observed

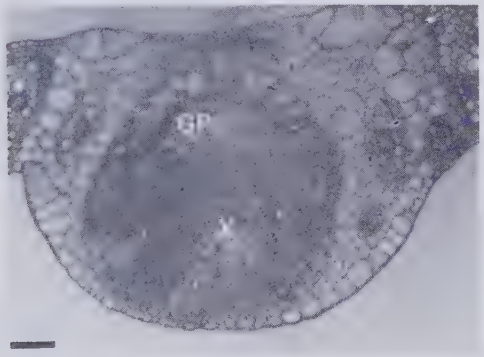

FIGURE 5. Cross section of a gall of Fiji disease showing proliferation of phloem (GP) and xylem (X). (Bar represents 125μ)

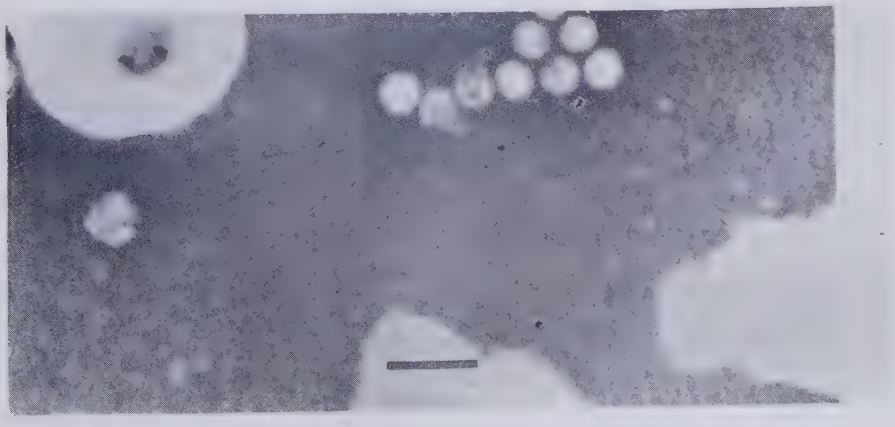

FIGURE 6. Partially purified virus particles from sep of maize plants used in assay for MWEV in affected Q90. (Bar represents 100mm).

with gall symptoms similar to those seen in sugarcane : *Brachiaria mutica, Eleusine indica, Panicum maximum, P. paludosum. E. indica.* These grasses appeared to be very susceptible, as plants were severely stunted, and the leaves were stiff and erect, with numerous galls.

DISCUSSION

The results indicated that the stunted stools and leaf galls in sugarcane in the Mulgrave area were caused by MWEV. However, these symptoms differed from those previously described by Grylls[2]. This is the first record of wallaby ear disease naturally infecting field grown sugarcane. The evidences supporting the thesis that MWEV caused the symptoms are as follows:

(a) *C. bimaculata* leafhoppers were present in large numbers in the original field where the disease occurred

(b) *C. bimaculata* leafhoppers, previously virus-free were able to produce wallaby ear symptoms in assay plants after feeding on diseased sugar cane

(c) the incubation period for the appearance of symptoms on assay plants was similar to that required for the development of maize wallaby ear disease symptoms, and

(d) the virus particles extracted from assay plants were similar to those obtained from MWEV-infected maize.

Unfortunately, no antiserum was available to test for serological relationship between the extracted particles and authentic MWEV. The failure to recover virus particles from extracts of diseased cane leaves is not contrary evidence, as it is known that recovery of virus from leaves of MWEV-infected maize is difficult.

Some of the symptoms produced by MWEV in sugar cane are like those caused by the Fiji disease virus. However, these two diseases can be distinguished relatively easily by the appearance and structure of the galls.

Wallaby ear disease appears to infect a number of hosts other than sugarcane. It occurs in several canegrowing districts in Queensland because of its generally mild effects on growth and the recovery of even the most severely infected plants.

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ENFERMEDAD "WALLABY EAR" DE LA CAÑA DE AZUCAR EN QUEENSLAND

C.C. Ryan, N.E. Grylls y L. Arkadieff

RESUMEN

En 1976 se encontro en diversos distritos productores de caña en Australia una enfermedad que causa formacion de agallas en la superficie abaxial de los hojas y enanismo de los retoños. Sintomas similares se observaron en yerbajos que crecian adyacentes a siembras de caña.

Las investigaciones demostraron que *Cicadulina bimaculata,* despues de alimentarse en la caña enferma, producia sintomas similares a los del "maize wallaby ear virus" (MWEV) en plantas de maiz. Particulas esfericas de tamano similar a las de MWEV (c. 45 mm), se visualizaron en estractos de plantas de prueba de maiz infectadas pero no en plantas de caña infectadas.

Este es el primer reporte de la enfermedad de "wallaby ear" ocurriendo naturalmente en caña de azúcar en el campo. La estructura de las agallas en la caña causada por MWEV y la enfermendad de Fiji fueron comparadas. Las agallas en la caña de azucar afectadas por MWEV estaban formadas mayormente por proliferacion de celulas de parenquima.

THE BIOLOGY OF *TUMIDICLAVA SP* (HYMENOPTERA: TRICHOGRAMMATIDAE), A PARASITOID OF THE SUGARCANE GIANT BORER *PHRAGMATOECIA GUMATA* SWINHOE

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ABSTRACT

The life cycle of *Tumidiclava sp.* from egg to adult emergence averaged 13.6 ±2.9 days. The mean longevity of starved and fed females was 3.4 ±0.5 and 3.5 ±0.9, and for males, 3.4 ±0.8 and 3.6 ±0.5 days, respectively. The mean fecundity of starved females with single and multiple copulation was 114.7 ±51.7, and 106.8 ±53.1, and for fed females it was 124.9 ±41.3 and 119.8 ±31.8, eggs respectively. The sex-ratio (♂ : ♀) and host-parasitoid ratio were 1.00 : 2.69 and 1.00 : 4.29. The mean percentage of parasitism and emergence were 66.4 and 88.1. This parasitoid seems to be monophagous.

INTRODUCTION

The sugarcane giant borer or Bandung borer *Phragmatoecia gumata* Swinhoe (Lepidopter; Cossidae) is widely distributed in the Malay region (Roepke[3]) Pan and Lim[2] reported that outbreaks of giant borer had occurred in Perak, Jahore Negeri Sembilan and had surveyed the potential for control by several species of parasitic Hymenoptera. The trichogrammatid wasp *Tumidiclava* sp is one of the important natural parasitoids of the giant borer and has become established in the Gula Perak Plantation.

This genus is a new record in Malaysia and has not yet been used for the control of sugarcane borers. This report is part of a series constituting a biological study of this species.

MATERIALS AND METHODS

A laboratory colony of *Tumidiclava sp* was established from adult parasitoids that emerged from *Phragmatoecia gumata* egg collected in Gula Perak plantations. Batches of newly-laid egg masses of the giant borer each containing 40 eggs were placed in test tubes (2 x 15 cm) and exposed either to a single-mated female or to a pair (♂ : ♀) permitting either single or multiple copulation. The test-tubes were covered with fine muslim held in place with rubber bands.

After 24 hours, all the parasitoids were removed and transferred to clean test tubes which contained 40 freshly-laid host eggs. This procedure was continued until the females died. A 10% honey solution was provided for the fed-parasitoid experiment.

The duration of the life cycle, from egg to adult emergence, longevity, fecundity, sex-ratio, host-parasitoid ratio, percentage of parasitism and emergence were recorded.

A survey of the host range was made by collecting other parasitized lepidopterous eggs from fields for emergence and examination in the laboratory. Light traps were used for catching living moths for eggs which were exposed to the parasitoids to find a suitable alternative host for laboratory rearing of the parasitoid. The experiment was conducted in the laboratory at a temperature of 28.5°C and R.H. 58.2%.

RESULTS AND DISCUSSION

Duration of life cycle

The life cycle of *Tumidiclava sp,* from egg to adult emergence, in the egg of *Phragmatoecia gumata* was completed in 13.6 ±2.9 days. (N = 4657) under the laboratory conditions. There are approximately twenty-seven generations per annum. There were no significant differences between the duration of male and female life cycles ($P > 0.05$).

Longevity of adults

The mean longevity of the ovipositing female was 3.4 ±0.5 days (r = 1-6, N = 1367) and 3.5 ±0.9 days (r = 1-7, N = 2039) under conditions of starvation and feeding, respectively. For the starved and fed males, the mean longevity was 3.4 ±0.8 days (r = 2-6, N = 658) and 3.6 ±0.5 days (r = 2-5, N = 608). The male lived slightly longer than the ovipositing female but there were no significant differences between the longevity of females and males ($P > 0.05$).

Fecundity

Fig. 1 shows the fecundity of the parasitoid with single and multiple copulation under starvation and feeding conditions. Estimates of daily oviposition, based on the emergence of adult progeny, showed that starved females exposed to single or multiple matings produced an average of 114.7 ±51.7 (r = 45-180) and 106 8 ±53.1 (r = 63-201) eggs, respectively. For fed females the corresponding figure were 124.9 ±41.3 (r = 71 − 200) and 119.8 ± 31.8 (r = 80 − 170) eggs. Fed females produced slightly more progeny than starved both in single and multiple copulation but none of the differences were statistically significant ($P > 0.05$). The number of eggs laid and the number host attacked decline with age. It was found that even the smallest host egg was capable of providing the nutrient necessary to permit the development of all the parasitoids laid in it, both in the laboratory and the field observations. However, the parasitoids that develop most quickly and appear on the 1st and 2nd

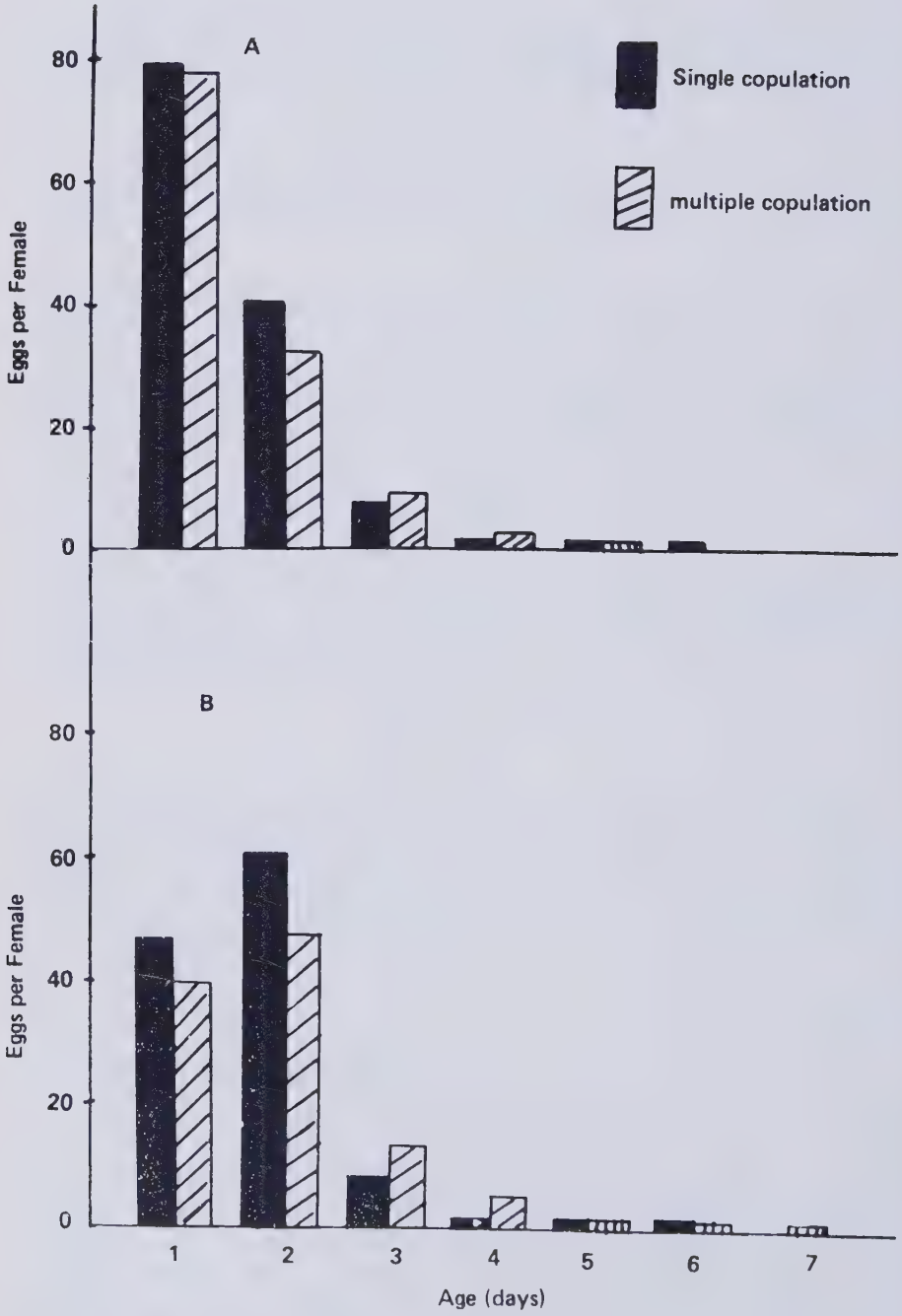

FIGURE 1. Daily oviposition of Fed (A) and starved (B) *Tumidiclava* sp with single and multiple copulations.

days of emergence are most active, while those which appear subsequently are weaker and less active. Oviposition lasted for 3-6 days. The total period of emergence of adult parasitoids from the host was 6-7 days.

The females commenced oviposition soon after hatching. No pre-oviposition period was observed. The females were never seen to feed at stab marks in the host eggs and they preferred to lay in host eggs 1-2 days old while they were still soft and fresh and did not attack the host eggs over 3 days old when they had hardened.

Sex-ratio and host-parasitoid ratio

Of the 4672 parasitoids which emerged from the 1089 parasitized eggs, 3406 individuals were females and 1266 were males. The sex-ratio (♂ : ♀) was therefore 1.00 : 2.69. The total number of female offspring produced by any one parent was always greater than that of the males. The average number of adult parasitoids emerging from a single host egg was 4.29 (r = 2.1 – 9.1) and usually contained both sexes.

Percentage of parasitism and emergence

The average percentage of parasitism obtained from batches of 40 eggs exposed to a single mated female was 66.4 (r = 38 – 100%, N – 40). The percentage of parasitized host eggs which gave rise to adult parasitoids, under laboratory condition was 88.1 (r = 78.4 – 100%).

Host-specificity

To determine the host range of *Tumidiclava sp,* a number of other parasitized lepidopterous eggs were collected from the fields, but none of them yielded any *Tumidiclava sp.* In addition, light traps were used for collecting living moths which were allowed to oviposit in the laboratory. The eggs were exposed to *Tumidiclava sp* in order to determine their suitability as hosts for rearing the parasitoids. The species tested were: *Spodoptera litura F.*, *Heliothis armigera* Hb; *Dasychiza sp; Amsacta lactinea* Cram, and *Creatonotus gangis* L. The silkworm in *Bombyx mori* L. and the rice moth *Corcyra cephalonica* Staint were also tested. No eggs were seen to be deposited in any of these hosts. Eggs of *Sesamia inferens* Walker were not observed to be attached by the parasitoid in the field, but have occasionally been parasitized in the laboratory, although percentage parasitism was low. It seems likely that *Tumidiclava sp* is naturally host-specific or monophagous.

Biotic potential available for biological control

An effective parasitoid is capable of destroying sufficient progeny of its host to reduce it to a low and relatively constant density (Flanders[1] 1939). As with other trichogrammatid parasitoids, *Tumidiclava sp* is an egg parasitoid and well suited to mass-production in the laboratory. Although the longevity of the adult is short, the fecundity is considerable. This species appears to

be host-specific and possesses an unusually long, strong, and flexible ovipositor compared with other trichogrammatids. The combination of these factors make this species promising as a biocontrol agent against the giant borer.

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LA BIOLOGIA DE *TUMIDICLAVA* SP (HYMENOPTERA: TRICHOGRAMMATIDAE), UN PARASITO DEL GIGANTE "BORER" DE CAÑA DULCE *PHRAGMATOECIA GUMATA* SWINHOE

Y. C. Pan y G. T. Lim

RESUMEN

Se presentan aqui los resultados de experimentos de laboratorio sobre la biologia de *Tumidiclava* sp. El ciclo de vida, desde hueva hasta adulto llevo un promedio de 136±2.9 dias. El medio de longevidad de hembras y macho o de hambreadas y nutridas fue 3.4±0.5, 3.5±0.9, 3.4±0.8 y 3.6±0.5 dias, respectivamente. El medio de fecundidad de hembras hambreadas y nutridas consimples y multiples copulaciones fue 114.7±51.7, 106.8±53.1, 124.9±41.3, y 119.8±31.8 huevas respectivamente. La razon (proporcion) de sexo (: DT) y la proporcion parasito-huesped fueron 1.00:2.69 y 1.00:4.29. El porcentaje de razon de parasitismo y emersion (salida) fue 66.4 y 88.1. Este parasito parece ser monofago.

BIOLOGY OF *PHRAGMATAECIA CASTANEAE* HUBNER, THE GIANT BORER OF SUMATRA, INDONESIA

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ABSTRACT

Phragmataecia castaneae Hubner is the dominant species of stem borer in sugar cane. Observations on its biology were made in the laboratory at an ambient temperature of approximately 32°C and a relative humidity of approximately 74%.

Data are provided on the headwidths of larval instars and on the duration of the immature stages. The mean duration of the life cycle from egg to adult was 107 days. The average number of eggs laid per female was 330. The potential finite rate of population increases per day is 1.0553.

INTRODUCTION

At least four species of lepidopterous stem borers are known affecting sugarcane plantings on newly cleared land in Sumatra. These are the pyralids *Chilo sacchariphagus* Boyer and *C. auricilius* Dudg., the noctuid *Sesamia inferens* Walk and the cossid *Phragmataecia castaneae* Hubner. The last mentioned species was determined by the Commonwealth Institute of Entomology.

P. castaneae is generally the dominant species in grown-up cane fields consisting more than 90% of the borer population. Although it is known from Europe to South East Asia, little seems to be known about this moth. This paper covers studies on the general biology of *P. castaneae* carried out in North Sumatra.

EXPERIMENTAL PROCEDURE

The experiments were conducted at Tanjung Morawa, 12 km from Medan. Sugarcane is grown in rainfed areas, on alluvial soils and an altitude of ± 20 meters. The average annual rainfall is 1810 mm.

Most of the work was carried out in the laboratory at an ambient temperature of 32.12 ± 0.79°C and a relative humidity of 74.87%. Eggs were placed in glass tubes. Freshly emerged larvae were maintained individually in vials containing leaf-sheaths of young cane stalks. Food was changed every two days. Pupae were placed individually in plastic tubes. Adult moths were kept in muslin cages containing dry cane leaves.

RESULTS

Egg

Freshly laid eggs have a creamish white color turning light brown after several days and becoming grey one or two days before hatching. Eggs are oval in shape, 1.8 mm long 0.8 mm thick (Fig. 1).

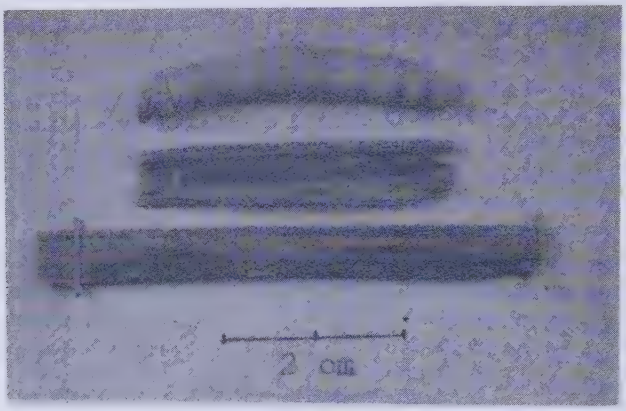

FIGURE 1. *Phragmataecia castaneae* Hubner., egg batch and larva.

Eggs were laid in patches consisting of one or more rows on the edges of old dry leaves still attached to the cane or on dead hearts leaves. The edges were then folded and secured to the leaf blades. A one centimeter row consisted of 9-12 eggs, depending on the way of attachment, i.e. either side by side or end to end. The longest observed row was 15 cm. The incubation period was 9-10 days.

Larvae

After hatching, the larvae grouped together for several hours around the egg patches. Then they dispersed by crawling, or became windborne on silken threads. Some larvae bored directly into the plants without dispersing. They entered the opening between the leafsheath (usually number + 3) and the stem. Three to seven days later the larvae bore into the stem. A single larva may destroy several nodes, averaging 2.28 ± 0.95 nodes before pupating inside the stem.

The color of a full grown larva is pinkish white with four reddish spot on each segment. Ten instars were observed during rearing (Table 1). On the basis of head-width, the growth increment between instars approximated to 1.11. The mean total duration of the instars was 80.04 ± 2.06 days. The length of a full grown larva may reach 5.5 cm for females and 4 cm for males.

The duration of the pre-pupal stage observed during the study was about two days.

TABLE 1. Headwidths and duration of the various instars of *Phragmataecia castaneae* Hubner.

Instar	Headwidths (mm)		Duration (days)	
	Mean	Range	Mean	Range
I	0.48	0.4 – 0.5	5.00	5
II	0.72	0.6 – 0.8	5.70	5 – 6
III	1.03	0.9 – 1.2	7.15	6 – 8
IV	1.31	1.2 – 1.4	7.45	6 – 9
V	1.67	1.5 – 1.8	7.05	6 – 8
VI	2.00	1.8 – 2.2	8.70	6 – 11
VII	2.40	2.2 – 2.5	10.55	7 – 14
VIII	2.61	2.4 – 2.7	9.15	7 – 12
IX	2.77	2.5 – 2.9	7.90	6 – 10
X	3.00	2.7 – 3.1	11.70	8 – 14

Pupa

The pupa is typically protected without the palpus maxilla and the proboscis is rudimental (Fig. 2). The spiracle of the first abdominal segment is not visible. At first the color is light yellow. After a few days the color gradually becomes darker until finally it turns to dark brown (Fig. 2).

FIGURE 2. *Phragmataecia castaneae* Hubner, moths and pupae (left: male; right female).

A few hours before emerging, the pupa wriggled itself to the exit hole. When the moth emerged, half of the pupal skin protruded outside. This habit distinguished it from the other lepidopterous borers. The average length of females and males

were 3.40 ± 0.21 cm and 2.53 ± 0.26 cm respectively, and the average duration of the pupal phase was 16.45 ± 1.13 days.

Adult

Emerging occurred during the afternoon. The moth, which is grayish brown in color has a rudimental proboscis. The antenna of the female consists of 45 annuli, which two third are bipectinate. Three fourths of the male antenna are bipectinate and the apical part is unipectinate.

Length of the female body was 3.02 ± 0.29 cm and that of the male was 2.18 ± 0.09 cm of 50 specimens examined, the average wing span of female and male were, respectively, 4.90 ± 2.77 cm and 3.75 ± 0.15 cm. To determine the potential egg-laying capacity, a record was made of the number of eggs laid and the number of ripe eggs or discernable oocytes which remained in the ovaries after the death of the moth. Females laid about 90% of the potential fecundity. In the laboratory, females survived only for about three days. The average number of eggs laid was 329.95 ± 47.68. The relationship between fecundity (Y) and pupal length (X) was shown in the following regression equation $Y = 227.5351 X - 364.5275$. The correlation coefficient $r = 0.7844$ was significant at 0.1% level.

Southwood[4] has used fecundity data to calculate the potential rate of increase of sugarcane borers. To calculate these values it was necessary to determine the maximum reproductive rate (Rm) which is equal to the mean maximum fecundity (F) per female and the fraction of females (f ♀) in the adult population. With a mean actual fecundity (not maximum fecundity as in Southwood) per female of 329.95 and a female fraction of 0.59, the maximum rate of reproduction was 194.67.

The potential rate of increase in the absence of mortality is expressed as $r = \log_e Rm/T$, where T is the sum of the mean duration of immature stages, pre-oviposition period and half of the egg laying period. $\text{Antilog}_e r_p$ is an expression of the potential finite rate of population increase per day. With a mean duration of the borer generation T = 99, the values of r_p and $\text{antilog}_e r_p$ is an expression of the potential finite rate of population increase per day. With a mean duration of the borer generation T = log, the values of r_p and $\text{antilog}_e r_p$ were 0.0484 and 1.0495 respectively.

DISCUSSION

The incidence of the giant borer affecting sugarcane plantings was first observed in 1976 on newly cleared land in Sumatra. Thompson[5], during his visit in Sumatra, considered this as the same species which badly attacked sugarcane in Malaysia, i.e., *P. gummata*. Specimens sent to the Commonwealth Institute of Entomology were identified as *Phragmataecia castaneae* Hubner. This species has never been recorded before in Indonesia. Kalshoven[2] mentioned only *Phragmatae-*

cia parvipuncta Hamps, infesting sugarcane in the mountains.

Little seems to be known about *P. castaneae* although it is known from Europe to Southeast Asia; in fact the Review of Applied Entomology has only four references to it since 1913 (Greathead[1]). While the life cycle of the larvae feeding on the common reed in Central Europe occupies two years, the life duration in Sumatra is much shorter, i.e. about 80 days.

In Lepidoptera, adult fecundity is usually related to the size of pupae (Williams[7]). Data obtained during the study revealed that the pupal weight can be used to predict fecundity. The value of r_p is comparable with those of Southwood[4], who obtained a r_p of 0.026 for *Castnia,* the giant borer of Guyana. The relatively smaller rate of potential finite daily rate of increase, compared to those of the pyralids, is the result of its longer life cycle.

The number of instars observed was ten. Larger numbers of instars occurred occasionally during insectary rearing. Pathak[3] has pointed out that the number of moults increases under stress conditions. The growth increment between instars based on head,width approximately to 1.1. This is less than 1.4 which is typical of many lepidopterous larvae (Wigglesworth[6]).

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BIOLOGIA DEL PERFORADOR GIIGANTE DE SUMATRA, INDONESIA, *PHRAGMATAECIA CASTANEAE* HUBNER

Boedijono Wirioatmodjo

RESUMEN

Phragmataecia castanea **Hubner es la especie predominante de perforador en la caña de azucar. Se realizaron algunas experiencias de laboratorio a temperatura ambiente ± 32°C, y humedad relativa de ± 74%. Este trabajo ofrece datos sobre las dimensiones de las cabezas de las larvas en los distintos estadios, asi como la duracion de vida de los adultos. El promedio del ciclo de vida del huevecillo al adulto fue de 107 dias. El promedio de huevecillos ovipositados por hembra fue de 330, y el aumento potencial de poblacion de dia 1.0553.**

ENTOMOFAUNA OF SUGARCANE IN MALAYSIA

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ABSTRACT

A preliminary list of insect pests associated with sugarcane and their arthropod natural enemies is presented based on collections made in 1970-1978 at sugarcane plantations in several states of Malaysia. A total of 360 species of insect pests and 120 species of natural enemies were recorded belonging to 98 and 34 families, respectively. Among them 32 species are considered as important pests of sugarcane.

Economic importance and control measures are briefly discussed.

INTRODUCTION

Sugarcane is one of the main crops in Malaysia. Plantations have been established in the States of Perak, Kedah, Johore, Perlis and Negeri Sembilan on land which was formerly deep primary jungle and planted with varieties of sugarcane which were imported from other cane growing countries.

In the tropics, the insect fauna is abundant in number of species and individuals and natural enemies are also numerous. A list of sugarcane insect pests was published by Corbet and Gater,[1] but it was incomplete and confined to a small area. The present survey of sugarcane pests and their arhropod natural enemies has been carried out in several states of West Malaysia from 1970-1978.

MATERIALS AND METHODS

The survey of sugarcane arthropod insect pests was made in large cane fields and on small-holder farms by sweeping, cutting open the stems of cane setts or sieving samples of soil from around the plants. A light trap was used for the collection of certain nocturnal species. Specimens collected were then brought back to laboratory for preserving or rearing.

To obtain the parasitoids, the parasitized hosts were collected from the fields and kept in an insectary until emergence. The predators were collected when observed attacking their hosts. The majority of the pests and their natural enemies was sent to the Commonwealth Institute of Entomology, London, United Kingdom for identification.

RESULTS AND DISCUSSION

As expected, the number of insect pests and their parasitoids and predators was found to be large and varied, many of which are first records or new species, while others still await determination. In Part I of the Results is listed the insect pests and in Part II, their parasitoids and predators.

Species found feeding on Sugarcane

Major pests are marked with an asterisk.
Where the genus contains more than one species, the number is given in bracket.

Foliage feeders

Orthoptera

Family: Acrididae

Acrida furrita L.
Acrida willemsei Dirsh
Atractomorpha psittacina de Haan
Catantops humilis Serv.
Catantops splendens Thunberg
Choroedocus illustris Walker
Erianthus guttatus Westwood
Gastrimargus marmoratus Thunberg
Homeopternis cinctifemus Walker
Oxyga hyla Serville
Patanga succincta L.
Phlaeoba antennate Brunner
Stenocatantops splendens Thunberg
Tagasta marginella Thunberg
Tetrix sp.
Traulia annadelei C. Bolivar
Trilophidia cinnulata Thunberg
**Valanga nigricornis* Burmeister
Xenocatantops humilis Serville

Family: Tettigoniidae

Ducetia japonica (Thunberg)
Hexacentrus sp.
Mecopoda elongata L.

Lepidoptera

Family: Arctiidae

Amsacta lactinea Cram.
Chicnaema perornata Walker
Creatonotus gangis L.
Creatonotus transiens Walker
Neasura apicalis Walker
Utethreisa lotrix Cramer

Family: Amathusiidae

Discophora timora perakensis Stickel

Family: Gelechiidae

? Autosticha sp.

Family: Geometridae

Hyposidra sp. ? *talaca* Walker

Family: Glyphipterigidae

Imma sp. ? *psoricopa* Meyr

Family: Heliodinidae

Eretmocera impactella Walker

Family: Hesperiidae

Telicota augias (L.)
Telicota bambusae (Moore) bina Evanas

Family: Limacolidae

Parasa bicolor Walker
Thosea sinensis Walker

Family: Lycidae

Trichalus communis Waterh

Family: Lymantriidae

Dasychira mendosa Hb.
Euproctis varians Walker
Orgyia turkata Butl
Psalis pennatula F.

Family: Lyonetiidae

Decadarchis sp. ? *Spodomicta* Meyr
Opogyona theobroma Bradl

Family: Noctuidae

Heliothis armigera Hb.
Leucania separata Walker
Spodoptera litura F.
Spodoptera mauritia Boisd

Family: Nolidae

? Nola sp.

Family: Picridae

Catopsilla pyranthe pyranthe L.
Melantis leda (L.) *ismene* Cr.

Family: Psychidae

Acanthopsyche sp.
**Clania variegata* Shell
Cremastopsyche pendula Joannis

Family: Syntomidae

Syntomis sperbius F.

Family: Tinaeidae

Tinea fraudulens Rosen
Tinea sp. near *Trita* Meyr
Tinea sp.

Family: Xyloryctidae

Procometia trochala Meyr

Family: Zygaenidae

Artona cataxantha Hmps.

Coleoptera

Family: Anthicidae

Anthicus sp.
Macratria fouqueti Pic.

Family: Anthricidae

Eucorynus crassicornis (F.)

Family: Bostrychidae

Sinoxylon anale Lesne

Family: Byrrhidae

Syncalypta sp.

Family: Chrysomelidae

Cassidinae

Aspidomorpha malaccana Spa.
Aspidomorpha milliaris (F.)
Epistictina viridimaculata Bhm.
Metriona catenata (Boh)

Chrysomelinae

Chalcolampra sedecium pustulata (Stal)

Criocerinae

Crioceris impressa (F.)
Crioceris semipunctata (F.)

Clytrinae

Aspidolopha bifasciata III

Eumolpinae

Pachnephorus sp.

Galerucinae

Aulacophora frontalis Baly
Madurasia quadrimaculata Jac.
? Monolepta sp.

Halticinae

**Altica* sp.
Aphthona sp.
Chaetocnemia basalis Baly
Chaetocnema sp.
Nisotra chrysomeloides Jac.

Hispinae

Agonita brittond Ubl.
Dactyeispa sumatrana Wse.
Monochirus moestus Baly
Gen. nr. *Promecotheca* sp.

Family: Coccinelidae

Epilachna indica Muls.

Family: Colydiidae

Bitoma siccana Psac.)

Family: Curculionidae

Blosyrus asellus Oliv.
Corigetus corbetti Mshl.
Gastero clisus binodulus Illig
Hypomeces squamosus F.

Family: Dytiscidae

Canthydrus flamomolatus Shp.
Canthydrus ritsemae Regt.

Family: Limnichidae

Byrrhinus sp.

Family: Melyridae

Idgis sp. ? *atriceps* Charp.

Family: Pselaphidae

Gen. & sp. ?

Dermaptera

Family: Chelisochidae

Proreus simulans (Stal)

Family: Forficulidae

Hypurgus humeralis (Kirby)

Psocoptera

Family: Archipsocidae

Archipsocus sp.

Family: Hemipsocidae

Hemipsocus sp.

The foliage feeders of sugarcane recorded comprised 101 species belonging to five orders and 37 families. These pests are often considered as of minor importance. Damage caused by these pests was slight and because of the mortality brought about by burning the sugarcane fields prior to harvesting, further control measures for these pests was unnecessary.

Sap feeders

Hemiptera

Family: Achilidae

Akotropis sp. ? *furmata* Mats
Ballomarius sp.
Callinesia sp.
Deferunda sp.

Family: Acleridae

Aclerda takahashii Kuw

Family: Aleyrodidae

Aleurolobus barodensis (Maskell)
Neomaskellia bergii Sign

Family: Aphididae

Aphis citricola v.d. Goot (= Spiraecola)
Aphis gossypii Glover
**Aphis maidis* (Fitch)
**Ceratovacuna lanigera* (Zehnt)
Hysteroneura setariae (Thos)
Longiunguis sacchari Zehnt

Family: Aphrophoridae

Clovia conifer (Walk)
**Clovia* sp. ? *sarawakana* Lall.

Family: Aradidae

Mezira triangulus Bergr

Family: Berytidae

Metacanthus pulchellus Dall

Family: Ceropidae

Callitettix versicolor (Fabr.)
Ectemnonotum bivittatum Lsp. & Ser.
Eoscarta sp.

Family: Cicadellidae

Amrasca sp.
**Balclutha* sp. (5)
Bothrogonia sp.
Cicadella spectra Dist.
Cicadella sp.

**Cicadulina bipunctata* (Mel) sp. ? *capitata* (Kirk)
Coelidai sp. *sen. Lat.*
Deltocephalus sp.
**Empoascanara* sp. (2)
Exitianus indicus (Dist.)
Exitianus sp.
Hecalus sp.
Inazuma dorsalis (Motsch)
Kolla mimica Dist.
Kolla sp.
Ledrotypa sp.
Litura unda Knight
Nephotettix apicalis Motsch
Nephotettix impicticeps Ishihara
Nephotettix nigropictus (Stal)
Nephotettix virescens (Dist.)
Paramesodes albinervosus (Mats) (2)
Petalocephalus sp. (1)
**Pruthiana* sp.
Thaia sp.
Xestocephalus sp. (1)
**Yamatotettix flavovittatus* Mats.
Gen. & sp. ? (22)

Family: Cixiidae

Sp. nr. *Caneirona indica* Dist.
Euryphlepsia sp.
Oliarus cocosivora Muir
Oliarus sp.

Family: Coccidae

Gen. & sp. ? (1)

Family: Colobathristidae

Phaenacanthe bicolor (Dist.)
**Phaenacantha saccharicida* Karsch
Phaenacantha sp.

Family: Coreidae

Anoplocnemis phasiana F.
Anoplocnemis sp. (2)
Homoeocerus serrifer Westw
Homoeocerus sp.
Leptocorisa acuta Thunb.
Leptocorisa chinensis (Dall)
Leptocorisa orotorius Thunb (Fabr.)
Leptocorisa rufomanginata (Fabr.)
Leptoglossus australis (Fabr.)
Leptortus linearis F.
Nictis sp.

Notobitus sp. ? *affinis* (Dall)
Riptortus sp.

Family: Delphacidae

Cemus sp.
Dicranotropis sp. (1)
**Eoeurysa flavocapitata* Muir
Nilaparvata lugens (Stal)
**Perkinsiella saccharicida* Kirk
Sogatella sp. (3)
Toya propinqua (Fieb)
Gen. nr. *Toya* sp.
Tropidocephala atrata Dist.
Tropidocephala festiva Dist.
**Tropidocephala saccharivorella* Mats.
Tropidocephala signata Dist.
Tropidocephala sp. (1)
Gen. & sp. ? (1)

Family: Derbidae

Cedusa sp.
Kamendaka sp.
Lamenia sp.
? Levu sp.
Perandenina sp.
Proutista moesta (Westw)
? Photana sp.
Zoraida sp. (1)
Gen. & sp. ? (1)

Family: Diaspididae

Aulacaspis madiunensis (Zehnt)
Greenaspis decurvata (Green)

Family: Dictyopharidae

Philotheria sp.
Thanatodictya fuscovittata (Stal)

Family: Flatidae

Family: Issidae

Lavane Consprese (Walk)
? Brahmaloka sp.

Family: Lophopidae

Lophops saccharicida Kirk

Family: Lygaeidae

Geocoris tricolor (Fabr.)
Germalus greeni Dist.

Pachybrachius nietneri (Dohrn)
Pachybrachius pacificus (Stal)
Pachybrachius sp.

Family: Meenoplidae

Nisia sp.

Family: Membracidae

Anchon ulniforme Buckt
Leptocentrus sp.

Family: Miridae

Creontiades sp.
Crytorhinus lividipennis Reuter
Deraeccoris sp.
Hallodapus albofasciatus (Notsch)
Proboscidecoris sp.

Family: Pentatomidae

Dalpada trimaculata Westw
Gestomus pygmacus Dall
Halyomorpha ficus F.
Megarrhamphus rostratus F.
Megarrhamphus sp.
Menida histrio (Fabr.)
Meygmenum brevicorne (Fabr.)
Nezara viridula (L.)
Phynchocoris humeralis (Thunb)
Scotinophara lurida Burmeister
Scotinophara coarctata Fab.
Tetroda sp.

Family: Plataspidae

Brachyplatys subaeneus Westw.
Coptosoma sp.

Family: Psuedococcidae

**Saccharicoccus sacchari* (CkII)

Family: Pyrrhocoridae

Dysderus megalopygus Breddin

Family: Ricaniidae

Ricania sp. (1)

Family: Tingidae

Cantacader sp.

Family: Tr piduchidae

Baruncides albosignata Dist.
Epora sp.
Ossoides sp.

Thysanoptera

Family: Thripidae

Fulmekiola serrata (Kobus)
Thrips ? flavidus Bagnall

Twenty-nine out of the 176 species of hemipterous sap feeders are considered as important pests.

None of the species of fulgorid and cicadoid causes direct damage of consequence to sugarcane, but several species of *Perkinsiella* and *Cicadulina* are vectors of Fuji and streak disease (Pemberton and Charpenter[5]) and are therefore of considerable potential importance.

Sugarcane wooly aphid, *Ceratovacuna lanigera* (Zehnt), mealybug *Saccharicoccus sacchari* (CkII), sugarcane whiteflies *Nemaskella bergii* Sign. and *Aleurolobus barodensis* (Maskell), sugarcane scale insects *Aclerda takahashi* (Kuw), *Aulacaspic madiunensis* (Zehnt) and *Greenaspis decurvata* (Green), frog-hoppers *Clovia* ? *sarawakana* Lall and *Clovia conifer* (Walk) and two species of sugarcane thrips *Fulmekiola serrata* (Kobus) and *Thrips* ? *flavipus* are normally kept under control by parasitoids and predators. Work on chemical control has not yet proved effective.

The outbreak of the colobathristid bug, *Phaenacantha saccharricida* Kansch has been reported in several states of West Malaysia (Lim and Pan[3]). Application of 0.07% endosulfan (Thiodan) was used in Kedah and Perlis for the control of this colobathristid bug (Tan and Johnson[6]).

Stalk borers

Lepidoptera

Family: Cossidae

**Phragmatoecia Castaneae* Hb.
**Phragmatoecia gumata* Swinhoe
**Phragmotoecia parvipuncta* Hmps
Phragmatoecia sp.
Zeuzera conferta Walker

Family: Noctuidae

**Sesamia inferens* Walker

Family: Pyralidae

Ancylolomia sp. ? *indica* F & R
Chilo incertellus Wlk ner Zak
Chilo indicus Kapur
Chilo polychrysa (Meyr)
**Chilo sacchariphagus* Bojer
Chilo terrenellus Sn. Rag
Chilotrala infuscatellus Sn.
**Emmalocera umbricostella* Rag.
? *Emmalocera* sp.
Scirpophaga rhodoproctalis Hmps
**Triporyza nivella* (F.)

Family: Tortricidae

**Tetramoera schistaceana* Snell

Coleoptera

Family: Curculionidae

**Calanda* sp.
**Rhodocnemis obscures* boisd
Gen. & sp. ? (2)

Family: Scolytidae

**Xyleborus fornicatus* Richh

Diptera

Family: Chloropidae

Gen & sp. ?

Family: Micropezidae

Mimegralla ? coerulifrons Macquert

At least 11 of the 25 recorded species of stalk or stem borers are listed as important pests. Some of these create serious problems and several others are potentially important. The relative importance of the borers in each state is different. Biological control of moth borers has been successfully established in Perak (Lim[2]). Control of stalk borers by *Trichogramma* continues to be satisfactory (Pan and Lim[4]).

Tumidiclava sp. (Hym : Trichogrammatidae) is used for the control of sugarcane giant borers *Phragmatoecia gumata* in Perak. In other states insecticides were applied for the control of these borers.

Dipterous borers are not serious pests although they occassionally attacked the seedlings.

The beetle borers are potentially important in certain areas. Field hygiene is stressed in the plantations as a means of reducing numbers.

Soil insects

Orthoptera

Family: Cryllidae

Anaxipha ? longealata Chopard
Brachytrupes portentosus (Lichtenstein)
Patiscus malayanus Chopard
Scleropterous coriaceus (Haan)
Teleogryllus consimilis (Walker)
Trigonidium cicindeloides humbetianum (Saussuro)

Family: Grylloptaipidae

Gryllotalpa africana Beauvois

Family: Oecanthidae

Oecanthus ? indicus Saussure

Tridactylus thoracicus Guerin

Isoptera

Family: Rhinotermitidae

Coptermes javanicus Kemner

Family: Termitidae

Globitermes sulphureus Hav.

Hymenoptera

Family: Formicidae

Acantholepis sp.

Anoplolepis longipes (Jerdon)
Campenotus sp. *(fulvomarginatus* group)
Crematogaster sp.
Meranoplus sp.
Polyrhachis proxima Roger
Tapinoma sp.
Tetraponera rufonigra (Jerdon)
Tetraponera sp.

Hemiptera

Family: Cicadidae

Dundubia rufivena Walk.
**Mausa* sp.
Platylomia sp.
Pomponia sp.

Coleoptera

Family: Anthicidae

Anthicus sp. (1)
Macratria fouqueti Pic.

Family: Cerambycidae Lamiinae

Pterolophia sp. nr. *robinsoni* Gah.

Cerambycinae

Chlorophorus annularis (F.)

Family: Elateridae

**Agryphus* sp.
**Heteroderes* sp.
Lacen sinensis Cand.

Family: Endomychidae

Parindalmus quadrilunatus (Gerst)
Trochoideus desjardinsii Guer

Family: Helodidae

Ora sp.

Seirtes pellucidus Waterh

Family: Hybosoridae

Ochodaeus xanthomelas (Wied)

Family: Inopeplidae

Inopeplus albonotatus (Mots)

Family: Monommatidae

Monomma sp. nr. *glaphysternum* Mars.

Family: Mordellidae

Hoshihananomia sp. ? *octoguttata* (Montr.)

Family: Nitidulidae

Cybocephalus sp.
Urophorus sp. nr. *foreicollis* (Murray)

Family: Phalacridae

Heterolitus thoracirus (Fleut)

Family: Scarabaeidae
Cetoniinae

Glycyphana festiva (F.)
Glycyphana sinuata (Wallace)
Protretia acuminata (F.)

Dynastinae

Alissonotum piceum (F.)
Clyster trachygygus Prell
Oryctes rhinoceros L.
Xylotropes gideon L.

Halticinae

Aphthona sp.

Melolenthinae

**Apogonia* sp. ? *expeditionis* Rits
Hoplia sp.
Lachnosterna sp.

Rutelina

**Adoretus compressus* (Weber)
Anomala cupripes Hope)
Anomala sp.
Parastasia confluens Westw

Fifty-eight species of soil insects, belonging to 20 families were recorded. The termites are important pests in cane fields. The usual treatment is application of γ- BHC after manually destroying the mounds. Damage to sugarcane by white grubs and wireworms has been observed but the occurrence of these pests is not yet widespread in Malaysia. Although the soil insect pests have to be recognized as injurious on the plantations, the damage they cause is at present so slight that

control measures have not far proved necessary.

Part II. The parasitoids and predators of sugarcane insect pests

Order/Family	Species	Host
Parasitoids		
Hymneoptera		
Aphelinidae		
	Aphelinus sp.	*Ceratovacuna langiera* (Zehnt) *Longiunguis sacchari* (Zehnt)
	Centrodora sp.	*Valanga nigricornis* Burmeister
	Encarsia sp.	*Aleurolobus barodonsis* (Maskell) *Neomaskellia bergii* Sign
Bethylidae		
	Rhabdepyris sp.	*Chilo sacchariphagus* Bojer *Tetramoera schistaceana* Snell
Braconidae		
	Apantales flavipes Cam.	*Chilo sacchariphagus* Bojer *Chilo sacchariphagus* Bojer
	Bracon chinensis Szeptligeti	*Sesamia inferens* Walker
	***Bracon hebetor* Say	*Chilotraea infuscatellus* Sn. *Emmalocera* sp. *Tetramoera schistaceana* Snell *Chilo sacchariphagus* Bojer
	Rhaconotus sp.	*Emmalocera* sp. *Triporyza nivella* (F.)
	Spathius hella Nixon	*Chilo sacchariphagus* Bojer
	Spathius sp.	*Chilo sacchariphagus* Bojer
Chalcididae		
	Brachymeria lasus (Walker)	*Chilo* sp.
	Brachymeria sp.	*Emmalocera* sp.

Encyrtidae		
	Anagyrus sp.	*Saccharicoccus sacchari* (Ckll)
	Aphidencyrtus sp.	*Ceratovacuna lanigera* (Zehnt) *Longiunguis sacchari* (Zehnt)
	Neoprochiloneurus sp. *Nikolskiella* sp.	*Aclerda takahashii* Kuw
	Ocencyrtus malayensis Ferriere	*Leptocorisa acuta* Thunb
	Trichomasthus sp.	*Aclerda takahashii* Kuw
Eucharitidae		
	Stilbula sp.	*Crematogaster* sp.

The parasitoids propagated in the Experiment Station for the control of sugarcane moth borers are marked with two asterisks.

Eulophidae		
	Tetrastichus sp. (Crino group) *Tetrastichus* sp.	*Eoeurysa flavocapitala* Muir
	Tetrastichus israeli (Mani & Kurian)	*Sesamia inferens* Walker
Eupelmidae		
	Anastatus sp.	
Formicidae		
	Oecoplylla smaragdina Fab.	*Chilo sacchariphagus* Bojer *Sesamia inferens* Walker *Tetramoera schistaceana* Snell
	Tetramorium guineense Fab.	*Phragmatoecia gumata* Swinhoe *Emmalocera* sp.
Ichneumonidae		
	Trophocampa sp.	*Parasara* sp.
	Vulgichneumon sp.	*Spodoptera* sp.
	Xanthocampoplex sp. nr.	*Phragmatoecia gumata* Swinhoe

	Nigromaculata (Cam.)	
	Xanthopimpla sp.	*Chilo sacchariphagus* Bojer *Sesamia inferens* Walker
Mymaridae		
	Anagrus optabilis (Perkina)	*Eoeurysa flavocapitata* Muir *Perkinsiella saccharicida* Kirk
	Anagrus sp.	*Saccharicoccus sacchari* (CkII)
Platygasteridae		
	Amitus sp.	*Neomaskellia bergii* Sign *Aleurolobus barodensis* (Maskell)
Pteromalidae		
	Promuscides? unfasciativentris Gir.	*Aclerda takahashii* (Kuw)
Scelionidae		
	Telenomus sp.	*Chilo sacchariphagus* Bojer *Emmalocera* sp.
	Telenomus sp.	*Leptocorisa* sp.
	Cryon flavipes (Ashm)	*Leptocorisa* sp.
Scoliidae		
	Scolia sp.	*Oryctes rhinoceros* L. *Xylotropes gideon* L.
Sphecidae		
	Liris aurata (Fabr.)	*Brachytrupes portentosus* (Lichtenstein)
Trichogrammatidae		
	***Trichogramma australicum* Girult	*Chilo sacchariphagus* Bojer
	***Trichogramma chilotraeae* Nagaraja & Nagarkatti	*Emmalocera* sp.
	***Trichogramma confusum* Viggiani	*Tetramoera schistaceana* Snell
	***Trichogramma japonicum* Ashmead	*Sesamia inferens* Walker

	)	
***Trichogrammatoidea nana*	)	
	)	*Phragmatoecia gumata Swinhoe*
(Zehnt)	)	*Phragmatoecia gumata Swinhoe*
	)	
	)	*Phragmatoecia castaneae* Hb
	)	*Phragmatoecia parvipuncta* Hmp s
	)	
	)	*Phragmatoecia* sp.

Diptera

Anthomyiidae

Anthomyia sp.	*Gastrimargus* sp.
Stenomicra sp.	*Gastrimargus* sp.

Tachinidae

Carcellia sp.	*Dasychira* sp.
Cuphocera varia F.	*Spodoptera* sp.
Plaexorista lucagus (Walk)	*Creatonotus gangis* L.
Pseudogonia rufifrons (Wield)	*Dasychira* sp.
Stomatomyia sp.	*Chilo saccariphagus* Bojer
Sturmiopsis inferens (Towns)	*Chilo sacchariphagus* Bojer *Sesamia inferens* Walker

Predators

Coleoptera

Carabidae

(Casnoided nigrofasciata (Sehn-G)	)	
	)	
Cryptocephalomorpha gaverei Rits	)	
Desera geniculata (Klug)	)	
	)	
Lachnothorax tokkia Gestro	)	Various insect pests
	)	
Lymnastis pilosus Bates	)	
	)	
Pheropsophus jessoensis Mor	)	
	)	
Planetes pendleburyl Andr	)	
	)	
Selina westermanni Mots	)	

Cicindelidae

Cicindela aurulenta F.	)	
Collyris diardi Latr. & Dej.	)	Various insect pests

Coccinellidae

Chilocorus nigritus (F.)	*Ceratovacuna lanigera* (Zehnt) *Aphis maidis* (Fitch) *Longiunguis sacchari* (Zehnt) *Hysteroneura setariae* (Thos) *Saccharicoccus sacchari* (CkII)
Chilocorus politus Muls.	*Aulacaspis madiunensis* (Zehnt)
	Greenaspic decurvata (Green)
Coccinella arcuata (F.)	*Ceratovacuna lanigera* (Zehnt) *Aphis maidis* (Fitch)
Coccinella repanda (Th.)	*Longiunguis sacchari* Zehnt)
Coelophora inaequalis (F)	*Hysteroneura setariae* (Thos)
	Ceratovacuna lanigera (Zehnt)
Cryptogonus fulvoterminatus Boh	*Aphis maidis* (Fitch) *Longiunguis sacchari* (Zehnt) *Hysteroneura setariae* (Thos)
Illeis bistigmosa Muls	*Saccharicoccus sacchari* (CkII) *Ceratovacuna* (Zehnt) *Aphis maidis* (Fitch)
Menochilus sexmaculata (F.)	*Longiunguis sacchari* (Zehnt) *Hysteroneura setariae* (Thos)
Pullus pallidocellis Muls	*Ceratovacuna laginera* (Zehnt) *Aphis maidis* (Fitch)
Pullus sp.	*Longiunguis sacchari* (Zehnt)
Rodolia sp.	*Hysteroneura setariae* *Saccharicoccus sachari* (CkII) *Allwolobus barodensis* (Maskell) *Neomaskellia borgii* Sign *Aphis maidis* (Fitch)
Scymnus sp. ? *nubilus* Huls	*Longiunguis sacchari* (Zehnt) *Hysteroneura setariae* (Thos) *Saccharicoccus sacchari* (CkII)

Spilocaria bissellata Muls.	*Ceratovacuna lanigera* (Zehnt) *Aphis maidis* (Fitch) *Ceratovacuna lanigera* (Zehnt)[1] *Longiunguis sacchari* (Zehnt)
Synonyocha Thunb	*Hysteroneura setariae* (Thos)
Staphylinidae	
Carpelimus peregrinus (Cam.)	*Chilo sacchariphagus* Bojer
Coproporus sp.	*Tetramoera schistaceana* Snell *Sesamia inferens* Walker *Emmalocera* sp.
Paederus sondaicus Fauv.	*Phragmaoecia gumata* Swinhoe
Philonthus aeneipennis Boh	*Triporyza nivella* (F.)
Dictyoptera	
Mantodea : Mantidae	
Tropidomantis tenera (Stal) *Statilia maculata* Thunberg	Various insect pests
Diptera	
Asilidae	
Philodicus chinensis Schiner	*Monolepta* sp.
Syrphidae	
Cephalochrysa maxima (Bezzi) sp. *demeijerei* Lindner *Eristalinus quinquestriatus* *Eumerus insistens* Curran *Paragus serratus* F.	*Aphis maidis* (Fitch) *Ceratovacuna lanigera* (Zehnt) *Hysteroneura setariae* (Thos) *Longiunguis sacchari* (Zehnt)
Hemiptera	
Anthocoridae	
Anthocoris nemorum (L.)	*Aphis maidis* (Fitch) *Ceratovacuna lanigera* (Zehnt) *Hysteroneura setariae* (Thos)
Blaptosthus sp.	*Longiunguis sacchari* (Zehnt)

		) *Eoeurysa flavocapitata* Muir
		) *Aphis maidis* (Fitch)
		)
		) *Ceratovacuna lanigera* (Zehnt)
	Xylocoris flavipes (Reut).	)
		) *Hysteroneura setariae* (Thos)
		)
		) *Longiunguis sacchari* (Zehnt)
Nabidae	*Alloeorthynchsus vinulus* Stal	)
	Nabis sp.	) *Eoeurysa flavocapitata* Muir
		)
	Prostemma fasciata Stal	)
Reduviidae	*Biasticus* sp.	)
		)
	Ectomocoris sp.	)
		)
	Ectrychotes annamensis Miller	)
		)
	Euagoras plagiatus Burn	)
		)
	Endochus cameronicus Miller	)
		)
	Gomesius hesionae (Kirk)D	)
	Harpagochares macilenta (Miller	)
	Homalosphodrus pendleburyi miller)	
	Oncocephalus sp. ? *modestus* Reut)	
	Petalochirus spinosissimus Dist.	) Various insect pests
		)
	Pirates sp. (1)	)
		)
	Polididus armatissimus Stal	)
		)
	Scipinia horrida Stal	)
		)
	Schidium marcidus (Uhler)	)
		)
	Sastrapada sp.	)
		)
	Sirthenea flavipes Stal	)
		)
	Sphedanolestes discifer Reut	)
		)
	Sycanus sp.	)
		)

Villus macrops (Walk)

Gen & sp. (♀)

Hymenoptera

Vespidae

Ropalida ? marginata (Lep) — *Melantis leda* (L.) *ismme* Gr.

Lepidoptera

Pyralidae — *Aphis maidis* (Fitch)

Isauria aphidivora Meyr — *Ceratovacuna lanigera* (Zehnt)

Hysteroneura setariae (Thos)

Longiunguis sacchari (Zehnt)

Neuroptera

Chrysopidae

Chrysopa sp. — *Aphis maidis* (Fitch)

Ceratovacuna lanigera (Zehnt)

Hysteroneura setariae (Thos)

Italochrysa sp. near *aequalis* — *Longiunguis sacchari* (Zehnt)

Walker — *Perkinsiella saccharicida* Kirk

Hemerobiidae

Aphis maidis (Fitch)

Micromus sp. — *Ceratovacuna lanigera* (Zehnt)

Hysteroneura setariae (Thos)

Longiunguis sacchari (Zehnt)

Mantispidae

Austroclimaciella quadrituberculata (Westwood)

Fifty two species of parasitoids belonging to 19 families and 68 species of predators belonging to 13 families were recorded in the survey.

The Malay Peninsula often provide conditions in which host plant, pest and natural enemy can all develop continuosly throughout the year so that there is no interruption in contact between host and natural enemy.

All trichogrammatid parasitoids have been established in the Sugarcane Experiment Station, Perak, for the control of the moth borers. Search for further natural enemies is in progress.

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ENTOMOFAUNA DE CAÑA DULCE EN MALASIA

G. T. Lim y C. Pan

RESUMEN

Se presenta una lista preliminar de insectos asociados con la peste de caña de azúcar y sus enemigos antropodos naturales basado en la colleción hecha en 1970-1978 en las fincas de azúcar en varios estados de Malasia. Se compeló una lista de 360 especies de insectos-pestes y 120 especies de enemigos naturales pertenecientes a 98 y 34 familias respectivamente. Entre estos, 32 especies se consideran como pestes importantes de la caña de azúcar.

Trata brevemente de la importancia económica y de medidas para su control.

FAUNA OF WHITE GRUBS IN SUGARCANE FIELDS OF OKINAWA AND THEIR RELATIVE IMPORTANCE AS PESTS OF SUGARCANE

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ABSTRACT

Extensive surveys of white grubs inhabiting in sugarcane fields of Okinawa counted 2 species of Cetoniidae, 8 species of Rutelidae, 7 species of Melolonthidae and 1 specie of Dynastidae. Of these, 15 species could be regarded as the pests of cane in different degrees of importance. *Anomala albopilosa* is most important among them in a real scale of numerical abundance and plant damage. Two species of large-sized melolonthid, *Melolontha masafumii* and *Tricholontha papagena* are also important although their occurrences are locally specified at present.

INTRODUCTION

In the past few years, outbreaks of *Anomala albopilosa* in several islets of Okinawa have continued to cause serious damage to sugarcane plants, especially in Miyako Is. and in some parts of Ishigaki Is. Other than this species, a few species of Rutelidae and Melolonthidae have temporarily occurred in abundance in some restricted localities and caused more than negligible damage to cane. Therefore extensive surveys were conducted to determine local faunas of white grubs and their relative importance.

EXPERIMENTAL PROCEDURES

Extensive underground samplings of white grub larvae in sugarcane fields were conducted on the main Okinawa Is., Miyako, Ishigaki, Iriomote and Yonaguni Islands. Except for those lacking keys for identification the collected larvae were identified as to species. When larvae keys were not available the larvae were reared in the laboratory until as adult stage for identification. Adults specimens caught by light traps and those collected on host plants were also used for confirmation of their local distributions.

Surveys were made in cooperation with Miyako and Ishigaki Branches of Okinawa Agricultural Experiment Station and the local Plant Protection Offices.

RESULTS AND DISCUSSION

Local distributions of white grub fauna together with relative importance of the respective species are summarized in Table 1. The relative importance was classified into the following six grades.

A: Outbreak type species (gradation or temporal types) causing serious damage to cane.
B: Species that are gradually increasing in number and probably cause serious damage upon reaching high density levels.
C: Species in latent number, causing light damage to cane.
D: Species of temporal bursts in number, but would not cause serious damage to cane.
E: Species that occur by chance in cane fields without causing any appreciable damage.
F: Species that entirely depend on organic matter in soil.

Takara[3], and Azuma and Oshiro[1] listed 8 species of white grubs as pests of cane. Of these, 7 species were identical. The remaining species were differently listed as *Apogonia bicarinata* by Azuma and Oshiro and *Anomala limbifera* by Takara[3], but distribution of the latter species in Okinawa is questionable. Thus seven species were newly added to those already listed.

At typical infestation areas, mixed populations of 2 or more species of white grubs were commonly seen in the same fields. For instances, *A. albopilosa, L. looshooana* and *Maladera* sp. in Miyako; *A. albopilosa* and *A. triangularias,* or *A. (Blitopertha) okinawaensis, T. papagena* and *A. albopilosa* in northern parts of the main Okinawa Is.; *A. albopilosa, A. esakii,* or *M. masafumii* and *A. albopilosa* in Ishigaki.

Short ecological notes concerning the five important species are as follows.

Anomala albopilosa (Fig. 1a)

This species is distributed throughout Okinawa except Kita- and Minami-Daito Islands, and is most important of all those listed in Table 1. The outbreak has been existing in Miyako since 1975. More than 90% of all cane fields there were infested in 1976-1977, and 39% of these were severely damaged by the infestation of 10 or more 3rd instar larvae per stool. Young instar larvae were reared on bagasse, but the final or 3rd instar larvae could not be reared on bagasse (Unpublished data). The 3rd instar larvae has a feeding stage and non-feeding stage. During the feeding stage, larvae feed voraciously on roots, internodes and buds. This feeding kills standing cane plants before harvest and causes failure of harvested stools to ratoon and of newly planted setts to grow.

TABLE 1. Fauna of sugarcane white grubs among islands of Okinawa and their relative importance as pest.

Species	Grade in relative importance**	Distribution among islands a	b	c	d	e	f
CETONIIDAE							
1. *Protaetia ishigakia* Fairmaire	D	O	O	O	O	O	O
2. *Oxycetonia jucunda* Faldermann	F	O		O	O	O	O
RUTELIDAE							
3. *Popillia lewisi* Arrow	E	O					
4.* *Anomala (Blitopertha) okinawaensis* Ohaus	D	O					
5.* *Anomala xanthopleura* Arrow	C	O	O		O	O	
6. *Anomala esakii* Sawada	A				O	O	O
7.* *Anomala albopilosa* Hope	A	O	O	O	O	O	O
8. *Anomala triangularias* Schonfeldt	A	O	O				
9.* *Anomala matsumurai* Sawada	B			O	O	O	
10.* *Adoretus sinicus* Burmeiter	C						
MELOLONTHIDAE				O	O	O	
11. *Maladera sp.*	B	O		O			
12.* *Apogonia shibuyai* Sawada	C	O	O				
13.* *Apogonia bicarinata* Lewis	C	O		O	O	O	
14. *Lachnosterna loochooana* Sawada	B	O	O	O	O	O	
15. *Granida schonfeldti* Brenske	E	O	O				
16. *Tricholontha papagena* Nomura	A	O					
17. *Melolontha masafumii* Nomura	A			O	O	O	
DYNASTIDAE							
18.* *Oryctes rhinoceros* Linne	C	O		O	O	O	O

Note: Letters from a *to* f *indicate Okinawa, Kume, Miyako, Ishigaki, Iriomote and Yonaguni islands, respectively.*

** Listed by Takara (1958) and Azuma and Oshiro (1967).*

*** For explanation, see text.*

The 3rd instar larvae usually occur from mid summer and gradually enter the non-feeding stage from late autumn to mid spring of the next year. Length of the feeding period is dependent on prevailing temperatures, but the non-feeding stage seems to be diapausing (Hokyo and Nagamine[2] and Unpublished data). Thus pupation occurs within a short period from mid-April to May, and adult populations peak in June. An estimation of adult mean longevity ranged from 15 to 25 days. Adults are nocturnal and many are caught by light traps. They fly to feed on young buds and leaves of various kinds of shade trees, maritime trees and weeds, and to oviposit in cane fields. Oviposition is made into the soil within 10 cm of the surface.

FIGURE 1a. *Anomala albopilosa*

FIGURE 1b. *Anomala esakii*

FIGURE 1c. *Anomala triangularias*

FIGURE 1d. *Tricholontha papagena*

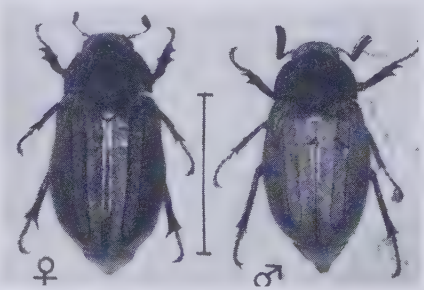

FIGURE 1e. *Melolontha masafumii*

Anomala esakii (Fig. 1b)

Distribution to this species is seen only in Ishigaki, Iriomote and Yonaguni islands. This species is rather similar to *A. albopilosa* in morphology and ecology. The body size of larvae and adults is somewhat larger than *A. albopilosa.*

Anomala triangularias (Fig. 1c)

This species is only seen in the main Okinawa Is. and Kume Is. A rapid increase in the population occurred in sandy soil areas of northern part of Okinawa Is. in 1978. Adults emerge earlier than *A. albopilosa* from late April to early May.

Tricholontha papagena (Fig. 1d)

Occurrences of this species are limited to some areas of northern part of Okinawa Is. Standing, dead cane plants occur before harvest when infested with 10 larvae or more per stool, and such damage has been caused frequently in cane fields of Motobu Peninsula since 1977. In contrast to the vertical infestation from roots upwards made by *A. albopilosa* larvae, this species attacked the underground parts horizontally. As a consequence, infested stools of cane lose their underground support. Adults begin to emerge from late October.

Male adults are attracted by light traps and collected in abundance from December to January. Female adults are probably negative in phototaxis.

Melolontha masafumii (Fig. 1e)

Occurrence of this species are only in rather limited parts of Miyako, Ishigaki and Iriomote Islands. Localized outbreaks occurred in Miyako and Ishigaki Islands in 1975 and 1976, respectively. The mode of infestation is similar to that by *T. papagena,* but both sexes of adults have positive phototaxis. They are caught in abundance by light traps from February to March. A female deposits a batch of *ca.* 30 egg per oviposition. Larvae of this species have a close resemblance to those of *T. papagena,* which causes difficulty in distinguishing between the two in the larval stage.

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FAUNA DE GUSANOS BLANCOS* EN CAÑALES DE OKINAWA Y SU RELATIVA IMPORTANCIA COMO UNA PLAGA DE LA CAÑA DE AZUCAR

Masaaki Nagamine

RESUMEN

Exploraciones extensivas de gusanos blancos que habitan en cañales de Okinawa, dieron como resultado la recoleccion de 2 especies de Cetoniidae, 8 especies de Rutelidae, 7 especies de Melolonthidae, y 1 especie de Dynastidae. De todas estas, 15 especies pueden ser consideradas como plagas de la caña de azucar en un mayor o menor grado de importancia. *Anomala albopilosa* es la mas importante en cuanto a abundancia y daño a la caña de azucar. Dos especies de gran tamaño, *Melolontha masafumii* y *Trichlolontha papagena,* son tambien consideradas importantes aunque su distribucion esta restringida en la actualidad.

*Gallina ciega — Nixticuil — gusano de manteca, etc.

CONTROL OF ANT DAMAGE TO POLYETHYLENE TUBES USED IN DRIP IRRIGATION SYSTEMS IN HAWAIIAN SUGARCANE FIELDS

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ABSTRACT

Ant damage to polyethylene tubes used in drip irrigation systems is a major problem on some of the Hawaiian sugarcane plantations. Damage can be controlled by reducing ant populations, by injecting insecticide into the drip irrigation system, by using ant-resistant tubes, and by utilizing agronomic practices. Mirex bait was currently used to control ant populations. Injecting heptachlor into the drip tubes at dosage of 75 g/1000 m of tube was effective in protecting the tubes from ant damage for at least 12 weeks. Tubes made of polyproplene or polybutylene material were less damaged by ants than tubes made of low-density polyethylene material. Thick-walled tubes (greater than 0.25 mm) often have fewer holes chewed through by ants on tube walls. Slit orifices were attacked less frequently by ants than the round orifices. Incorporating toxic chemicals into tube material could eliminate ant damage for more than 3 months. A short, frequent watering schedule was prefered to a schedule with long periods of watering and shutdowns.

INTRODUCTION

The Hawaiian sugar industry had converted approximately 19,000 ha of sugarcane land from furrow irrigation to drip irrigation by the end of 1978. One of the major problems we have encountered in drip irrigation is that ants chew through the tube walls and enlarge the orifices along the length of the tube. This changes the hydraulic characteristics of the system, causing, a reduction of water pressure resulting to an uneven water distribution. If the problem is not corrected, it could result in heavy yield losses in some parts of the field due to poor plant growth caused by severe water shortage. With an increasing number of fields converted to drip irrigation in Hawaii, the problem of ant damage to drip tubes becomes a serious threat to the well-being of the industry.

Three ant species in Hawaiian sugarcane fields cause the damage then are the fire ant, *Solenopsis geminata* (F.), the big-headed ant, *Pheidole megacephala* F).,

and the Argentine ant, *Iridomyrmex humilis* (Mayr.). All of these ants make their nests in the soil or partly in the soil. The fire ant is the most destructive but its distribution is limited to hot, dry and low lands. It nests often in sunny, open areas, among short grasses or in partial shade. Within a sugarcane field, fire ants can be found anywhere when the leaf canopy is open. Ten months after planting however, more fire ant nests are located near the field margins, usually less than 15 m from the border areas (Chang *et al.*[2])

The big-headed ant is widely distributed in shady and moist areas, often nesting near the soil surface under the trash or in old sugarcane tools. They remain in sugarcane fields throughout the crop season and can caused extensive tube damage when their population is high.

The Argentine ant is dominant in areas generally above 150 m in elevation. We have not observed severe tube damage caused by this ant species.

Ant damage to drip tubes can be reduced by the following approaches:

(1) Reducing ant populations
(2) Establishing a toxic barrier around the orifices
(3) Using ant-resistant drip tubes
(4) Utilizing agronomic practices

This report covers our work on these approaches. It discusses the problems we encountered in conducting the study and gives some of the results we obtained.

MATERIALS, METHODS AND RESULTS

Reducing Ant Populations In Sugarcane Fields

An obvious answer to the problem of ant damage to drip tubes is to reduce the ant population in the field. Insecticides for ant control in crop lands have been applied in two ways – by baits and broadcast applications to the soil.

Mirex Granulated Bait 300 (0.3% mirex, 15% soybean oil) on a corn or maize cob grit carrier was used for reducing ant populations in drip-irrigated sugarcane fields. It gave effective and economical control, but the registration for use of Mirex was cancelled by the United States Environmental Protection Agency (EPA) on December 1, 1977, with a provision that stocks on hand after that date could still be used until exhausted. A new bait by American Cynamid, AC 217300, is under study and may be used in the future if the chemical is proven effective and economical.

Of the residual soil insecticides tested to replace Mirex bait, the most effective ones were the chlorinated hydrocarbons such as chlordane, aldrin, dieldrin and heptachlor. However, these insecticides were all being phased out of used by the EPA. None of the other short-residue pesticides already registered for used on

sugarcane such as diazinon and endosulfan (Thiodan) were effective.

We have always been cautious in using broadcast application of insecticides in Hawaiian sugarcane fields because of potential harmful effects on natural enemies of many sugarcane insect pests. Negm and Hensley[6] showed that after suppression of the ant population by soil surface treatment with heptachlor, damage by *Diatraea saccharalis* (F.) was significantly increased. However, we do not preclude experiments to explore the use of certain newer pesticides such as the synthetic pyrethoids.

We prefer to use bait to reduce ant populations because it is specific, easy to apply, and a small amount of active insecticide is needed on a per area basis. For instance, in using Mirex bait, only 8 g/ha of active material was applied at a cost of about U.S. $10.00/ha. Because of this small amount, there was a minimum effect on other non-target insects and beneficial insects. This fitted in well, with our overall insect pest management program.

Establishing A Toxic Barrier Around The Orifices

Most of ant damage to the thick-walled (over 0.36 mm) drip tubes occurs at the orifices, which can be protected by injecting a contact insecticide into tubes that are buried in the soil. The insecticide is injected into the water while the irrigation system is in operation and saturates the soil around the orifices to establish a toxic barrier to keep ants away.

We have evaluated the effectiveness of a number of insecticides in preventing ant damage to drip tubes. The results showed that tubes treated with heptachlor at a dosage of 75 g/1000 m of tube had no orifice enlargement after 12 weeks of exposure, while control tubes had 23% and 13% of the orifices enlarged by the fire ant and the big-headed ant, respectively. Registrations of heptachlor on food crop except for uses under specified exemption were cancelled in February 1978 by the U. S. EPA. We have been granted registration for local use of heptachlor by the Hawaii State Department of Agriculture. We have not conducted tests with heptachlor over an entire crop cycle of 24 months. Nevertheless, we believe that by re-treating the fields at proper intervals, heptachlor will provide adequate protection of the drip tubes until the sugarcane crop is harvested.

The use of heptachlor as an alternative to Mirex baits to control ant damage to drip tubes is a temporary answer. We do not look at heptachlor as a permanent solution because of its potential adverse effects of the environment and other non-harmful organisms (especially beneficial insects). Other insecticides showing promise are the synthetic pyrethroids such as fenvalerate and permethrin at a dosage of 30 g/1000 m tube. These insecticides are less persistent in the environment and should have fewer problems being registered for crop use by the EPA. However these are not as effective as heptachlor and are more expensive.

Another method of establishing a toxic barrier is by soaking the drip tubes in solutions of insecticide or insect repellent. Results showed that tubes soaked for 3 hours in 0.4% diesel oil solution of fenvalerate had 1% of the orifices enlarged by the fire ants after 3 months exposure in the field. Twenty percent of the orifices were enlarge on the untreated tubes. Unfortunately, it is not possible to re-treat the tubes with this method also, 3 months of protection is not adequate since the average harvest age for Hawaiian sugarcane is 24 months. We have discontinued studying this method of establishing a toxic barrier.

Ant-resistant Tubes

A. Factors affecting resistance of tubes to ant attack

Many types of drip irrigation tubes from different manufacturers are currently available on the market. Each type is of different material, construction, wall thickness, orifice configuration, and orifice size. We compared some of these attributes for their roles in ant resistance.

1. Type of material, We found that polypropylene and polybutylene materials are most resistant to ant damage than the low-density polyethylene material. The orifice diameters on polypropylene material were enlarged to only ¼ and ½ of the sizes of orifices on polyethylene material by the fire ant and the big-headed ant, respectively, after 7 days of exposure. Work with other insects also showed that polypropylene is more resistant to penetration, than polyethylene [3]. At present, however, virtually all tubes installed on Hawaiian plantations are polyethylene.

2. Wall thickness. The wall thickness of drip tubes currently available commercially ranges from 0.13 to 0.76 mm. We found that ant damage decreases with increasing wall thickness. The fire ant enlarged the orifice diameter of 0.15-mm-thick polybutylene material to 616% of its original size in 7 days, while the orifice diameter on 0.73-mm-thick material was only enlarge 32% during the same period. Also, ants often chew directly through the tube wall if the thickness is less than 0.25 mm. Increasing the thickness of plastic materials has been reported to reduce termite damage in a similar manner (Gay and Wetherly[4]).

3. Orifice configuration. Tube manufacturers use either a laser beam or a mechanical punch to make tube orifices. Result of tests in our laboratory showed that orifices made by a mechanical punch or by a cold needle had only ½ to 1/3 the amount of ant damage to the orifices made by a heat process (Chang and Ota[2]).

   The orifice shape also makes a difference. We found that slit orifices, as on the T-Tape tubes (T-System Corporation, San Diego, California), had less ant damage than round orifices. Field results showed that the slit orifices of T-Tape had no enlargement vs. 29% of the round orifices enlarge after 139 days in a sugarcane field infested with big-headed ants.

This was possibly due to the fact that, while slit orifices remain closed when the tube was not used for irrigation, round orifices ramained open. The ants walked by slit orifices without detecting them; whereas they investigated and probed into round orifices. Also, closed orifices made it difficult for ants to insert their mandibles to initiate chewing. They have no difficulty with the open, round orifices.

B. Incorporation or impregnation of plastic with chemicals

Polyethylene-blend drip tube material can be made more ant resistant by incorporating chemicals. Chang and Ota[2] also reported that certain organic tin compounds were highly effective in reducing ant damage. More recently we have incorporated the insecticides heptachlor, bendiocarb, fenvalerate permethrin, and propoxur into the low-density polyethylene materials. The preliminary field results showed that material incorporated with 0.5% by (weight) fenvalerate had no orifice enlargement vs 457% increase for orifices on the untreated material after 104 days in naturally occurring fire ant nests in the field. However, the results from different trials were in consistent due to difficulties in mixing chemicals into the hot (300°C) polyethylene material in our laboratory.

Kirkhill Rubber Company (Brea, California) has produced three tube materials with toxic chemicals, for us to test for ant resistance. One of these materials was undamaged by ants after being buried in naturally occurring ant nests in the field for 104 days. In contrast, the orifice diameter of untreated polyethylene-blend tube material was increased 352% and 453%, respectively, by the fire ant and the big-headed ant. We are hopeful that tubes made up to the latter material will be available for further field tests.

Gay and Wetherly[4] and Beal *et al*[1] incorporated insecticides and inert fillers into PVC plastics hoping to increase termite resistance. Gay and Wetherly[4] showed that the only materials without any termite attack were the ones incorporated with aldrin and dieldrin. PVC materials incorporated with 5% hard silicon, zircon flour or diatomaceous earth could reduce termite attack to only 66-80%.

Agronomic Practices to Reduce Ant Damage

Chang and Ota[2] showed that tubes buried or in constant darkness had significantly more ant damage than tubes placed on the soil surface and subjected to continuous light. Ants would be unlikely to attack hot tubes laid on the soil surface under the sun, whereas a cool buried tube could be attacked day and night. However, the difference in ant damage may not be significant after the closing of the leafy canopy.

The sugar plantations in Hawaii do not irrigate the fields continuously because of water shortages. Instead, drip irrigation systems are run at intervals. Ants cannot attack the orifices when the irrigation system is operating. Thus, a

watering schedule of short, frequent irrigation rounds will result in less ant damage to tubes than one of long watering periods (over 2 days) and long shut-downs (Chang and Ota[7]).

DISCUSSION

At present, we do not know why ants chew polyethylene tubes. Chang and Ota[2] indicated that ants are attracted to water and to low concentrations of CO_2 which could be released at the orifices from microbial activity on organic matters brought by irrigation water into the tube. Hangartner[5] showed that CO_2 also elicited a digging response in the fire ants. It was possible that ant enlarge the orifices to gain access to the tube interior in search for food or water, or a nesting site, especially since we have noticed ant broods in the tubes. However, we still cannot explain why the ants chew the polyethylene tube wall. Ants usually initiate the attack on the wall areas with surface blemishes. Beal *et al*[1] reported that smoothing the surfaces of the material was about as effective as doubling thickness in reducing termite perforations. However, Gay and Wetherly[4] showed that the physical nature of the surface of a plastic is of no importance in determining the liability of the plastic to termite attack.

In our opinion, the best solution to reduce or eliminate ant damage to drip tubes is the development of an ant-resistant tube which relies basically on physically and hydraulic characteristics. For instance, the most commonly used tube in Hawaii has a wall thickness about 0.56 mm and double-wall construction, so that they hydraulic characteristics of the tube are more tolerant to ant-damage orifices. Using this tube, it has been unnecessary to treat for ants in many fields that do not have the fire ant. However, the tube cannot withstand damage by the fire ant or high populations of the big-headed ant and insecticide treatments are required.

Incorporation of chemicals into the tube material will continue to be explored. Recent developments indicate that new candidates of potential chemicals used in conjunction with modern plastic technology may make it feasible to develop tubes that are resistant to ants. However, we must still consider the safety problem in manufacture and handling, the cost, and the process of registration and approval by the U. S. EPA.

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EL CONTROL DEL DAÑO DE HORMIGAS A TUBOS DE POLIETILENO EN EL SISTEMA DE RIEGO POR GOTEO EN CANALES DEL HAWAII

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RESUMEN

El daño de las hormigas a los tubos de polietileno usados en el riego por goteo, es un problema serio en ciertas plantaciones de caña del Hawaii.

El daño puede controlarse al reducirse la poblacion de hormigas, ya sea mediante la inyección de insecticidas en el sistema de riego, usando tuberia resistente, o utilizando ciertas practicas agronomicas. Mirex* es el producto que normalmente se usa en Hawaii para el control de las hormigas. Al inyectar heptacloro en las tuberias, a dosis de 75 g/100 m. de tuberia, se protegen los tubos del daño de las hormigas por lo menos durante 12 semanas. La tuberia de polipropileno o polibutileno es mas resistente al ataque de las hormigas que las de polietileno de baja densidad. Los tubos de paredes gruesas (mas de (0.25mm) presentan menos daños. Los orificios de salida longitudinales, en forma de rajaduras, son menos atacados que los redondos. Al incorporarse material toxico en la materia prima componente de la tuberia, se elimina el daño por mas de 3 meses. Un programa de riegos cortos y frecuentes es mas aconsejable que periodos largos de riego e inactividad.

*Insectidia de tipo hidrocarburo clorinado.

INTEGRATED CONTROL OF SUGARCANE BORERS IN THE NORTHWEST PENINSULA MALAYSIA AND LAMPUNG, SUMATRA, INDONESIA.

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ABSTRACT

By use of suitable cultural and biological methods, the sugarcane borer population had been brought under control and their damage on cane minimized. Average borer internodes damage had been reduced from 15% to 5% in Northwest Malaysia, and from 45% to 7% in Lampung, Sumatra within a period of three years.

Attempts had been made to define the ecosystem, the biotic and abiotic factors contributing to the borer population fluctuation under local conditions. The biological control agents viz. *Trichogramma* **spp., *Apanteles flavipes* Com., and *Diatraeophaga striatalis* Tns., played an active role in limiting the borer population increase. Use of insecticide was minimal.**

INTRODUCTION

Sugarcane borers were the major pests in the cane growing regions in Northwest Peninsula Malaysia and Lampung, Sumatra, Indonesia. Outbreak of borers occurred in both regions during the initial stage of plantations development.

In Northwest Peninsula Malaysia, the sugarcane plantation is located in the State of Perlis in an area bordering Thailand with a total of 10,000 ha. Development of the cane area started in 1969. Outbreak of borers occurred during 1971 to 1973 with 20 - 30% internode infection on susceptible varieties F 134 and Q 58, and 15% internode infection on tolerant variety NCo 310. The borer species were *Proceras sacchariphagus* Hmps (*Chilo saccharaphagus* Boj), *Eucosma isogramma* and *Sesamia inferens* Walk.

In Indonesia, the plantation is located in the province of Lampung on Sumatra island with a total area of 18,500 ha. Development of the area started in 1975 and is scheduled for completion in 1980. Planting of cane for seed started in August 1975 and a total of 185 ha were planted by the end of the year. The initial

seed canes were mostly obtained from Java. A serious outbreak of borers occurred the following year (Fig. 1). During the drought from May to August 1976, many of the 7 - 8 months old cane had more than 80% internodes infected. The average internode infection of the plantation was increased from less than 1% in March 1976 to 45% in September 1976. The borer species were *Chilo sacchariphagus* Boj., Chilo auricilius Dodg. and *Scirpophaga nivella* F. The heavily infested fields were burnt and discarded. This drastic action created an adverse shortage of seed cane and subsequently held back the planting schedule between October 1976 and February 1977. The second outbreak of borers occurred in September 1977. About 900 ha of canes planted in mid-1976 adjacent to the nursery area suffered 45% internodes infection (average for plantation 17%). These canes were burnt and ratooned.

In Java, Ruinard[1] had worked out the index and magnitude of damage caused by stem borers (*Chilo sacchariphagus* Boj., *Chilo auricilius* Dodg.); the loss factor for every internode bored, reduced pol % juice by 0.125%. For top borers (*Scirpophaga nivella* F.), the magnitude of damage had been correlated to sugar loss (Pers Comm[2]). The percentage sugar loss for 5%, 15% and 25% infestation were 0.4%, 2.1% and 8.9% respectively.

In view of the high monetary loss caused by borer damage and the favorable local conditions for the pest establishment, an integrated control program was initiated to suppress the high borer population.

MATERIALS AND METHODS

1. *Introduction of biological control with both exotic and indigenous parasites.*

Three species of egg parasites; *Trichogramma australicum* Gir., *Trichogramma japonicum* Ashm. and *Trichogrammatoidea nana* were mass propagated in the laboratories of Perlis and Lampung Plantations using eggs of *Corcyra cephalonica* Staint. as media and released in highly infested fields.

Two larval parasites; *Diatraeophaga striatalis* Tns. and *Apanteles flavipes* Com. were also mass propagated in the laboratory of Lampung Plantations using cane borers as media. The borers were mainly obtained from the laboratory and supplementary supply from field collection. Breeding of *Chilo sacchariphagus* Boj., and *Chilo auricilius* Dodg. *in vitro* had been practiced in Lampung Plantation to provide media for propagating of these parasites. These parasites were released in the highly infested fields after mating.

Other parasites not successfully propagated in the laboratory were collected from field and released to field where the population of the parasites were low.

Collection of borer eggs and larvae from fields was done regularly to determine the parasitism rate and establishment of parasites after mass release.

2. *Study on the seasonal fluctuation of borer population and damage.*

Survey of borer infestation rate and checking of borer population density of different instars were done by taking random samples every month at various zones in the plantation. The integrated control program was prepared according to the result of this study.

3. *Study on the effect of field practice on borer control.*

Selection of borer-free seed pieces for planting and roguing of infested shoots to reduce borer population was practiced. Burning of cane before harvesting and trash burning during ratooning to destroy borers was also practiced. Setts were treated with insecticide to eradicate borers which escaped from seed selection.

4. *Study of sex attractant.*

Synthetic sex pheromone vials obtained from England were used to trap the male moth of *Chilo sacchariphagus* Boj. and to study moth population and the potential of a sex attractant as part of the borer integrated control program.

RESULTS AND DISCUSSIONS

Biological Control

Trichogramma australicum Gir., *Trichogrammatoidea nana* and *Trichogramma japonicum* Ashm. were isolated from cane fields outside the new development area in Perlis Malaysia in 1972 and successfully propagated in the laboratory. Mass release of the parasites was started in the following year. Initially only the heavily infested fields received the parasites and the results were very encouraging with field parasitism rate increased from 0 to 80% within 6 months. The parasitism rate had been maintained in the range of 45% to 70%. Approximately 9×10^6 parasitic wasps were propagated and released in the fields covering 2,000 ha of cane fields monthly.

The egg parasites *Trichogramma australicum* Gir., *Trichogramma japonicum* Ashm. and *Trichogrammatoidea nana* were introduced from East Java to the sugarcane plantation in Lampung Sumatra for propagation in the laboratory in February 1976. In May 1976 the egg parasites were mass-released into the seed nursery where borer infestation rate was high (45% internode infection). In the initial experiment stage, 90% parasitism rate had been achieved by the parasites. Over two years of intensive and large scale release of the egg parasites, the borer population had been reduced to a great extent which subsequently contributed tremendously to the lowering of borer internode damage percentage on cane.

In the middle of 1978, *Diatraeophaga striatalis* Tns. and *Apanteles flavipes*

Com., the larval parasites, were both introduced into the biological control program in Lampung. They have been successfully propagated in the laboratory and mass-released into the fields. The parasitism rate in the released fields has continued at 20% to 30%. On top of these, a great assortment of natural enemies had been recovered from parasitized borers and eggs from the fields. Seven *Diptera* and *Hymenoptera* species had been identified from parasitized stem borer eggs, larvae and pupae; and five species of *Hymenoptera* from parasitized top borer eggs and larvae in cane fields (Table 1).

TABLE 1. Local parasites of stem borers and top borers found in South Sumatra

Borers	Parasites
Stem borers:	Egg parasites:
1. *C. auricilius*	1. *Trichogramma* spp.
2. *C. sacchariphagus.*	2. *Telenomous* sp.
	Larval parasites:
	1. *Apanteles* sp.
	2. *Camphyloneurus* sp.
	3. *Sarcophagidae.*
	Pupal parasites.
	1. *Gambroides* sp.
	2. *Xanthopimpla* sp.
Borers	**Parasites**
Top borers:	Egg Parasites.
1. *Scirpophaga nivella*	1. *Telenomous* sp.
	2. *Trichogramma* spp.
	3. *Tetrastichus* sp.
	Larval parasites:
	1. *Stranobracon* sp.
	2. *Elasmus* sp.

The attempts to breed the rest of the local parasites in Lampung were not successfull as no suitable host has been found. However, the more predominant species e.g., *Telenomous* sp. and *Stenobracon* sp., for top borers were collected in their larval or pupal stages, then released to highly infested fields where the parasite population was low, to insure a more widespread occurrence of the parasites in the whole plantation (Table 2)

In principle, the breeding and release programs of the biological control

agents was arranged and practiced according to the information obtained from the basic studies under the local conditions (Table 3).

TABLE 2. Field parasitism rate of *Telenomous* spp. on borers in 1978

Month	Parasitism rate on stem borer (%)	Parasitism rate on top borer (%)
January	—	36.8
February	7.1	20.3
March	27.5	20.7
April	8.0	17.0
May	6.4	44.6
June	8.7	32.4
July	0.0	42.3
August	0.3	36.4
September	0.9	29.4
October	9.0	54.7
November	0.0	33.9
December	1.1	40.1

TABLE 3. Principles of breeding and releasing program

Parasites	Preferred host	Life cycle of parasites	No. parasites per ha	Age of cane to be covered (month)	Frequency
Trichogramma spp.	*C. auricilius*	8 days	3500	3rd	1
	C. sacchariphagus			4th	2
				5th	2
				6th	1
				7th	1
Apanteles flavipes	*C. sacchariphagus*	24 days	100	5th	1
				7th	1
Diatraeophaga striatalis	*C. auricilius*	26 days	30 pairs	6th	1
				8th	1
				9th	1

Seasonal Fluctuation of Borer Population and Their Damage.

It was observed that heavy borer infestation were often associated with dry weather conditions. In Perlis Malaysia, the dry season starts in late December and ends in April. In Lampung Sumatra it starts in May and ends in October. For the rest of the months the canes in both areas are rainfed; growth is usually retarded during dry season and the canes succumbed to borer attack. The borers tunneled into the cane stalks and tunneled toward the growing points; consequently higher percentages of "dead top" and side-shooting resulted from borer attack during the dry season.

Surveys conducted in Lampung Sumatra in November 1977 after the dry season on the heavily infested fields indicated that 63% of the canes of 1404 ha surveyed suffered more than 20% "dead top" and side-shooting (Table 4). It was found that 94% pf the "dead top" resulted from attack by borers (mainly stem borers), only 4% caused by drought and 2% by disease such as Pokkah boeng.

It was also observed that borer larvae was more abundant in the dry months. In the weakened stalks, more than 1 borer per stalk could be found. A record of 21 borers (*Chilo auricilius* Dodg.) were collected from a stalk of variety Ps 8.

TABLE 4. Distribution of canes showing "dead-top" and side-shooting.

Variety	Ha surveyed	% dead-top / side-shooting							
		10	10–20	20–30	30–40	40–50	50–60	60–70	70+
NCo 310	847	84	332	241	167	30	0	10	10
Ps 41	530	26	76	133	32	38	77	61	87
Total	1404	110	408	374	199	68	77	71	97
%	100	7.8	29.0	26.6	14.2	4.8	5.5	5.1	6.9

Effect of Field Practice on Borer Control

1. *Planting borer-free seed cane.*

Borers were able to survive in setts after being buried in the planting furrows for 2 weeks, thus the movement of seed cane is one of the main ways to spread the pests. To reduce the spread of borers to new fields, setts were careufully selected and then treated with 0.2% Diazinon insecticide prior to planting. The treatment killed most of the larvae that escaped from visual inspection and selection.

2. *Roguing*

Roguing was done in an attempt to reduce the borer population, especially that of top borers *Scirpophaga nivella* F., The borers and eggs collected were used to determine the field parasitism rate from released. parasites. Roguing was carried out at 2-week interval in fields ranging from 2 to 4 months of age. The efficiency of the roguing operation was evaluated by checking the percentage of borers obtained from rogued plants and the percentage of infested cane left in the fields.

Results of roguing were satisfactory. It is estimated that a total of 9.4×10^3 larvae per hectare was removed from fields.

With an average of 2.5% tiller infestation. Roguing helped in reducing the pest population during the initial stage of the crop.

3. *Effect of burning on borer population.*

Burning of cane before harvesting is commonly practised in both plantations in Perlis Malaysia and Lampung Sumatra. Surveys were conducted to determine the survival rate of borers in the harvested fields throughout the first harvesting season in Lampung Sumatra. Burning before harvesting caused some mortality but did not give good control. Survival rate of borers in cane stalks was very high, averaging 71% in fields after burning for harvest (Table 5) This might have been due to the occassional poor burn of the cane. Even after burning trash in fields (Table 6) after harvest a substantial number of borers (19%) still survived.

TABLE 5. Borer survival rate in cane stalks after burning for harvest.

	No. of borers checked			
Borers	Total	Dead	Lived	Survival rate
Chilo auricilius	890	269	621	69.8 %
Chilo sacchariphagus	3271	903	2368	72.4 %
Average				71.1 %

4. *Chemical control.*

Furadan, a systemic insecticide was effective against top borers when Furadan 10G was injected near the growing point of infested stalks (Budiono[3]) however, the application was considered too laborious and time-consuming, and high cost of basal application of the insecticide does not warrant its use on a large scale.

Experiments carried out in Lampung Sumatra using various doses of Monocrotophos and Methidathion spray at 3 week intervals to control stem/top borers, showed no significant difference between treatments and check plots. Experiments

TABLE 6. Borers survival rate in cane tops left in field after and before trash burning in harvested field.

Type of harvesting	No. of borers			
	Total	Dead	Live	Survival rate
Burnt cane [a]	837	506	331	39.5 %
Green cane [b]	267	145	122	45.7 %
Burnt cane [c]	209	169	40	19.1 %

Note: a + b were checked before *the trash was burnt, while c were checked* after *the trash was burnt.*

carried out in Perlis Malaysia using Thiodan (endosulfan) and Diazinon to control stem borers were not successful.

Chemical controls were not feasible against cane borers. Time of application is critical in applying contact insecticides as only brief periods of exposure of borers would be encountered within its life cycle.

Sex Attractant

Preliminary trials were conducted in Lampung Sumatra cane fields in the use of synthetic sex attractant which is specific for male moth of *Chilo sacchariphagus* Boj. obtained from Tropical Product Institute, London. The chemical was dispensed in small plastic vials each containing 500 microgrammes of the attractant. The vial was used in conjunction with a water trap (Campion & Nesbitt[4]). The results were excellent as the vials showed effective attraction up to 15 weeks, and one of the vials trapped 191 male moths of the *Chilo sacchariphagus* Boj. in one evening. The total catches for 8 weeks reached as high as 661 moths. It is too early to conclude whether the sex attractant will contribute to reducing the borer infestation in the fields surrounding the traps. Knowledge of the radius of effective attractancy of the pheromone is yet to be determined.

From information obtained in the population density and age structure survey 2 weeks before the set up of traps, it was found that if there were a high population density, a high number of catches in the first week was experienced. Age of canes does not affect the catches as long as the traps were set above the cane canopy. It is believed that if enough traps were erected in one field per hectare for a sufficiently long period, the borer population or infection rate could be successfully reduced. It is possible that the borer population and infestation rate can be estimated or forecasted with reference to the number of catches over a certain period.

The aforementioned integrated control practices had effectively reduced the damage and loss incurred by borers in Perlis Malaysia and Lampung Sumatra sugar cane plantations. In Perlis Malaysia sugarcane plantation, damage caused by stem borers in 1972 averaged 15% internodes infected with the highest rate at 30%. Infestation rate had been reduced and maintained to less than 5% internodes in 1975. In Lampung Sumatra sugar cane plantation, average internode infection by borers was 45% with many fields suffering from more than 85% internodes damage in 1976. Infection rate had been reduced to 12% damage in July 1978 and dropped further to 7% in January 1979 (Fig. 1).

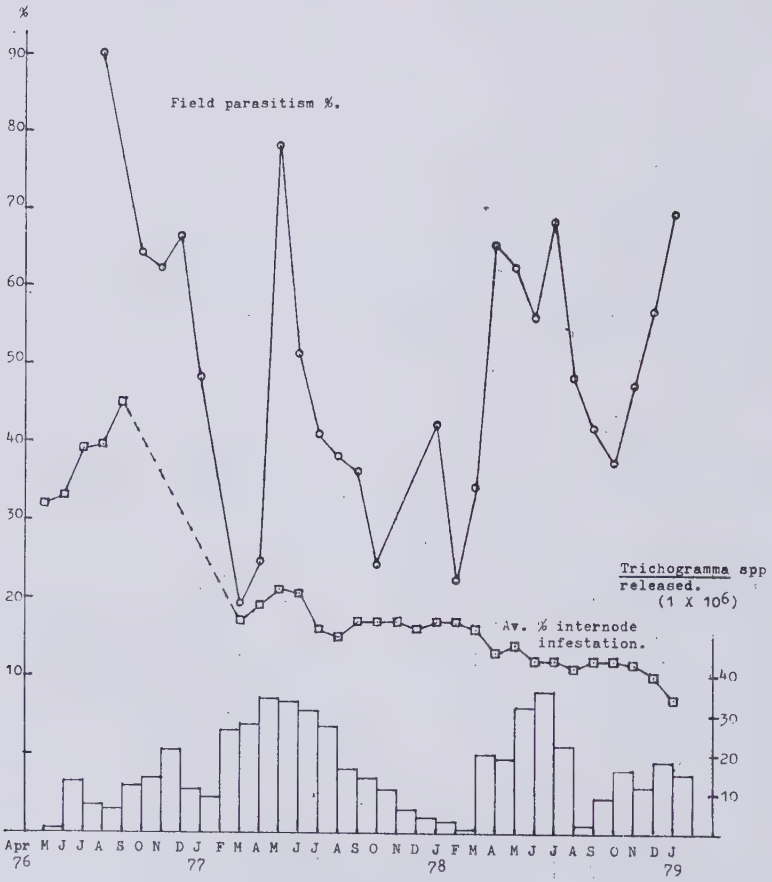

FIGURE 1. The effect of *Trichogramma* spp. on stem borers in Southern Sumatra.

CONCLUSIONS

In the past, people had relied too much on insecticides for control of borers. Owing to its tunnelling habit in the canes, most of the insecticides do not reach the

pest in sufficient quantity to kill the borers. Sugar cane is an extensive crop, to completely control the borers, great amount of insecticides are needed to cover the whole plantation, either by aerial spraying or basal applications. However, these are very expensive operations and large-scale applications of insecticides often cause environmental and social problems – pollution, development of resistance and resurgence of target and minor pests, and elimination of natural enemies, which upset the whole natural balance. The adverse effect is enormous.

Exploitation and artificial manipulation of natural factors and introduction of appropriate cultural practices will be adequate to cope with the borer problem in sugar cane plantations in northwest peninsula of Malaysia and Lampung Sumatra.

Dry seasons magnify the damage of borers on cane, however, it is during this period when most of the cane is harvested. The timely removal of cane and hence the borers will reduce the borer population and subsequently the internode infection rate.

Many other field practices such as burning before harvest, clearing trash from harvested fields, planting clean cane and roguing infested cane all help to a great extent to reduce the borer problem and restrict their spread to other areas.

Valuable information has been obtained regarding the local ecological conditions, the borer population age structure, absolute population density and distribution patterns, and the occurrence of natural enemies. The biological control programmes were based upon the information obtained. The laboratory propagated parasites were mass released at regular intervals in fields according to their location, cane age, infestation rate and borer population structure. Following the releases the parasitism rate of the released parasites was evaluated. This provided useful information regarding their performance in the field.

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CONTROL INTEGRADO DE LOS BARRENADORES DE LA CAÑA DE AZUCAR EN EL NOROESTE DE LA PENINSULA DE MALAYSIA Y LAMPUNG, SUMATRA, INDONESIA

S. W. Tan y H. L. Koh

RESUMEN

Los barrenadores eran los principales plagas de la caña de azucar en el noroeste de la Peninsula de Malaysia y Lampung, Sumatra, Indonesia. Mediante el uso de metodos culturales y biologicos appropiados la poblacion de barrenadores se ha controlado, y el daño a la caña ha disminuido considerablemente. Mediante estos metodos el promedio de entre-nudos (cañutos) perforados se ha reducido de un 15 a un 5 porciento en el noroeste de Malaysia, y de un 45 a un 7 porciento en Lampung, Sumatra, en un periodo de 3 anos.

Se ha tratado de aclarar el sistema ecologico, o sean, los factores bioticos y abioticos que contribuyen a las fluctuaciones de las poblaciones de barrenadores bajo condiciones locales. Los agentes de control biologico, como *Trichogramma* spp., *Apanteles llavipes* Com., y *Diatraeophaga striatalis* Tns., han jugado un papel muy activo en evitar el aumento de poblacion de barrenadores. El uso de insecticidas fué minimo.

NATURAL CONTROL OF *DIATRAEA SACCHARALIS* (FABR. 1794) EGGS IN SUGARCANE FIELDS OF SAO PAULO

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ABSTRACT

Extensive data (from May 1975 to December 1977) was accumulated on the fate of sugarcane borer (*Diatraea saccharalis* F.) eggs in several commercial cane fields, to determine the rates of natural control. Freshly laid eggs were placed among sugarcane leaves, simulating as far as possible, a natural situation. They were checked periodically until their complete maturation or destruction. Results show an average of 76.16% natural control, with differences between locations and years. Predators appeared as the major component of this natural control (63.64%), while parasites take account of only 3.90% of eggs, and the remaining 8.36% is attributed to other causes (possibly adverse climate, mechanical damage, disease, and even sucking predators). Actual rates of parasitism and predation by sucking insects may have been higher as chewing predators destroyed both healthy and parasitized eggs.

INTRODUCTION

Populations of the sugarcane borer, *Diatraea saccharalis* (F.) are usually controlled by many natural factors which reduce their rate of increase in sugarcane fields. These natural control agents affect practically all borer stages.

The mode of action of some of these factors, especially the larval parasites, have been studied extensively, but other natural control factors have been studied less. Some work done in Louisiana showed borer predators as being very important natural control factors (Negm and Hensley[3], Charpentier *et al*[1]), even more important than larval parasites.

It is very difficult to obtain quantitative information on natural control of other borer stages. Studies and written information regarding eggs are almost always concentrated on their parasites, especially *Trichogramma*. Attempts have been made to use them for biological control of the borer (Metcalfe and Breniere[2]). Meanwhile, other natural enemies of borer eggs, especially predators, have been shown to be much more important in Louisiana (Negm and Hensley[3]).

The need for quantitative information to improve borer population management schemes motivated this study to quantify the role of all natural control components of borer eggs, under commercial canefield conditions.

EXPERIMENTAL PROCEDURE

Fresh borer egg masses obtained from mass rearing were used. Egg masses were obtained from insectaries located in Sao Jose and Barra Grande Mills and from those of Copersucar's experiment stations located in Jau and Piracicaba.

Egg masses were conditioned on paper strips. These are paper pieces with 2 to 4 eggs masses, marked with a circle and with a number indicating the total eggs in each mass, as shown in Fig. 1. Details of these paper strips were recorded in special blank forms prepared for this study. These records allowed identification of eggs masses that may have been completely destroyed by chewing predators.

The paper strips were attached to cane leaves to simulate natural oviposition (Fig. 2). These strips were distributed in commercial cane fields. The cane field characteristics were also recorded.

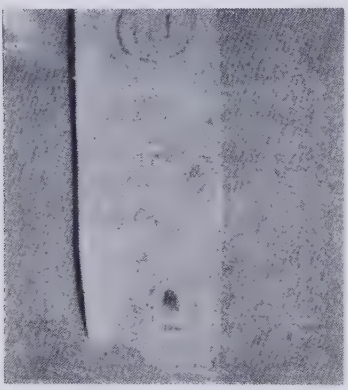

FIGURE 1. Egg masses conditioned on paper strips.

FIGURE 2. Paper strips attached to cane leaves to stimulate natural oviposition

Regular visits were made to determine the fate of the egg masses. If all egg masses of any strip were destroyed, it was removed and the fate of eggs recorded. The other egg masses, still in good condition, were retained in the field until the embryos reached "black head" stage (ready to hatch). At this stage, they were then taken to the insectary and kept until they hatched or until parasite emerged. After a reasonable time all eggs which had not yet yielded borers or parasites were considered to be "non-hatched" or non-viable.

Following the above procedure, paper strips were distributed fortnightly in the same cane field of each location.

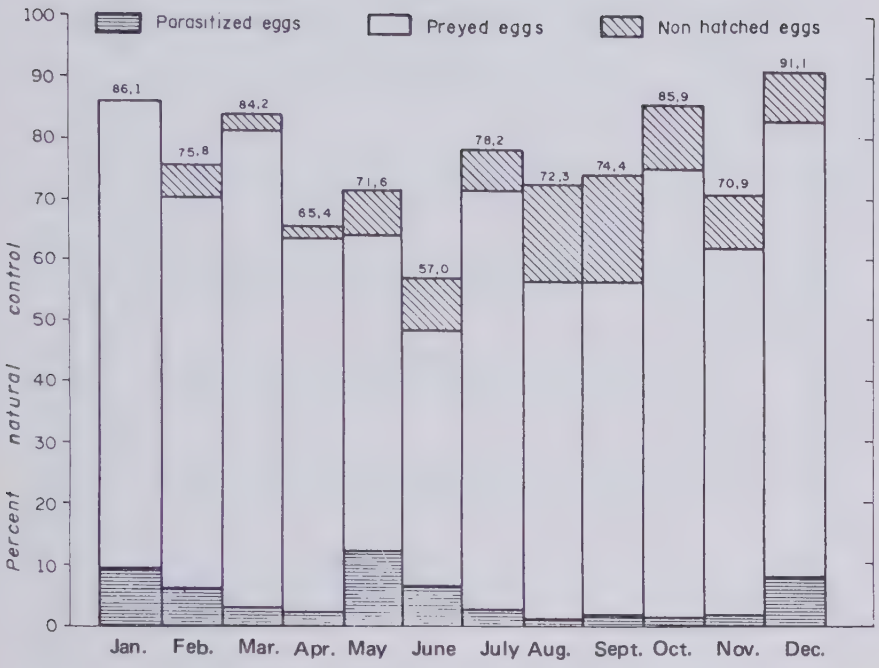

FIGURE 3. Natural control of parasitized *D. saccharalis* eggs.

RESULTS

A summary of results is presented in Table 1 and graphically illustrated in Fig. 3. These results show an average 76.16% natural control, without well-defined variations among months. Variations are well marked among the 4 locations (Tables 2 to 5).

Since white coloured strips could affect natural control, some comparative tests were run with egg masses oviposited on white and green paper. Results are shown in Table 6.

Table 7 shows results of natural control of borer eggs exposed in plant and ratoon canes.

DISCUSSION AND CONCLUSIONS

Results in Sao Paulo differ somewhat from those found in Louisiana (Negm and Hensley[3]). Average egg predation in Sao Paulo was higher and chewing predators destroyed much more eggs than sucking predators, differing from data of

TABLE 1. Natural control of *Diatraea saccharalis* eggs, in the State of Sao Paulo, Brazil. Weighed averages of studies carried out in some sugarcane fields from May 1975 to December 1977.

Month	Exposed masses	Exposed eggs	Parasitized eggs		Preyed		Eggs		Non hatched eggs		Natural control	
			No.	%	Chewed	Sucked	Total	%	No.	%	No.	%
JANUARY	261	2655	243	9.15	1962	81	2043	76.95	0	0.	2286	86.10
FEBRUARY	210	2152	131	6.09	1326	58	1384	64.31	116	5.39	1631	75.79
MARCH	254	2771	80	2.89	2042	134	2176	78.53	78	2.81	2334	84.23
APRIL	284	3047	65	2.13	1652	229	1881	61.73	46	1.51	1992	65.38
MAY	364	3757	462	12.30	1672	267	1939	51.61	291	7.75	2692	71.65
JUNE	266	2698	184	6.82	1018	104	1122	41.59	232	8.60	1538	57.01
JULY	440	4800	119	2.48	2837	461	3298	68.71	335	6.98	3752	78.17
AUGUST	250	3487	25	0.72	1881	62	1943	55.72	555	15.92	2523	72.35
SEPTEMBER	333	3463	53	1.53	1636	265	1901	54.89	624	18.02	2578	74.44
OCTOBER	405	4285	47	1.10	2858	314	3172	74.03	464	10.83	3683	85.95
NOVEMBER	402	4641	57	1.23	2697	119	2816	60.68	415	8.94	3288	70.85
DECEMBER	320	3055	241	7.89	2180	116	2296	75.16	248	8.12	2785	91.16
TOTAL	3789	40811	1591	3.90	23761	2210	25971	63.64	3520	8.63	31082	76.16

TABLE 2. Natural control of borer eggs in cane fields of Sao José. Average records from May 1975 to December 1977.

Month	Exposed masses	Exposed eggs	Parasitized eggs		Preyed		Eggs		Non hatched eggs		Natural control	
			No.	%	Chewed	Sucked	Total	%	No.	%	No.	%
JANUARY	100	895	105	11.73	663	81	744	83.13	0	0.	849	94.86
FEBRUARY	48	455	116	25.49	165	58	223	49.01	116	25.49	455	100.00
MARCH	84	991	66	6.66	616	134	750	75.68	67	6.76	883	89.10
APRIL	148	1521	35	2.30	887	223	1110	72.98	12	0.79	1157	76.07
MAY	168	1578	323	20.47	7.39*	143*	882	55.89	111*	7.03*	1316*	83.40*
JUNE	139	1804	184	10.20	497*	96*	593	32.87	163*	9.04*	940*	52.11*
JULY	257	2864	70	2.44	1780*	345*	2125	74.20	258*	9.01*	2453*	85.65*
AUGUST	59	784	16	2.04	478	39	517	65.94	99	12.63	632	80.61
SEPTEMBER	90	807	0	0.	405	171	576	71.38	132	16.36	708	87.73
OCTOBER	147	1367	0	0.	678	217	895	65.47	342	25.02	1237	90.49
NOVEMBER	94	919	19	2.07	508	55	563	61.26	272	29.60	854	92.93
DECEMBER	116	1070	96	8.97	634	83	717	67.01	137	12.80	950	88.79
TOTAL	1450	15055	914	6.07	8050*	1645*	9695	64.60	1825	12.12	12434*	82.59

**partial figures*

TABLE 3. Natural control of borer eggs in cane fields of Copersucar's experiment Station Jaú. Average records from August 1975 to December 1977.

Month	Exposed masses	Exposed eggs	Parasitized eggs		Preyed		Eggs		Non hatched eggs		Natural control	
			No.	%	Chewed	Sucked	Total	%	No.	%	No.	%
JANUARY	87	791	138	17.45	417	0	417	52.72	0	0	555	70.16
FEBRUARY	65	533	15	2.81	335	0	335	62.85	0	0	350	65.67
MARCH	89	981	14	1.43	627	0	627	63.91	11	1.12	652	66.46
APRIL	88	999	30	3.00	350	6	356	35.64	134	13.40	420	42.04
MAY	97	821	39	4.75	274	100	374	45.55	134	16.32	547	66.63
JUNE	52	306	0	0	150	0	150	49.02	55	17.97	205	66.99
JULY	102	992	0	0	328	86	414	41.73	49	4.94	463	46.67
AUGUST	90	1154	0	0	748	0	748	64.82	220	19.06	968	83.88
SEPTEMBER	171	1519	53	3.49	607	31	638	42.00	378	24.88	1069	70.38
OCTOBER	85	699	0	0	359	0	359	51.36	109	15.59	468	66.95
NOVEMBER	121	1271	9	0.71	726	64	790	62.16	138	10.86	937	73.72
DECEMBER	109	961	136	14.15	635	33	668	69.51	17	1.77	821	85.43
TOTAL	1156	11027	434	3.94	5556	320	5876	53.29	1145	10.38	7544	67.61

TABLE 4. Natural control of borer eggs in cane fields of Copersucar's experiment Station Piracicaba. Averages records from October 1975 to November 1976.

Month	Exposed masses	Exposed eggs	Parasitized eggs		Preyed		Eggs		Non hatched eggs		Natural control	
			No.	%	Chewed	Sucked	Total	%	No.	%	No.	%
JANUARY	59	689	0	0	683	0	683	99.13	0	0	683	99.13
FEBRUARY	78	838	0	0	668	0	668	79.71	0	0	668	79.71
MARCH	81	799	0	0	799	0	799	100.00	0	0	799	100.00
APRIL	48	527	0	0	415	0	415	78.75	0	0	415	78.75
MAY	28	240	0	0	240	0	240	100.00	0	0	240	100.00
JUNE	49	249	0	0	249	0	249	100.00	0	0	249	100.00
JULY	27	272	0	0	272	0	272	100.00	0	0	272	100.00
AUGUST	63	963	0	0	307	0	307	31.88	212	22.01	519	53.89
SEPTEMBER	35	470	0	0	278	0	278	59.15	0	0	278	59.15
OCTOBER	140	1798	47	2.61	1505	77	1582	87.99	1	0.06	1630	90.66
NOVEMBER	139	1686	0	0	1102	0	1102	65.36	5	0.30	1107	65.66
DECEMBER	80	805	9	1.12	795	0	795	98.76	0	0	804	99.88
TOTAL	827	9336	56	0.60	7313	77	7390	79.16	218	2.34	7664	82.09

TABLE 5. Natural control of borer eggs in cane fields of Barra Grande sugar mill. Average records from May 1975 to February 1976.

Month	Exposed masses	Exposed eggs	Parasitized eggs		Preyed		Eggs		Non hatched eggs		Natural control	
			No.	%	Chewed	Sucked	Total	%	No.	%	No.	%
JANUARY	15	280	0	0	199	0	199	71.07	0	0	199	71.07
FEBRUARY	19	326	0	0	158	0	158	48.47	0	0	158	48.47
MARCH	–	–	–	–	–	–	–	–	–	–	–	–
APRIL	–	–	–	–	–	–	–	–	–	–	–	–
MAY *	71	1118	100	8.94	419	24	443	39.62	46	4.11	589	52.68
JUNE *	26	339	0	0.	122	8	130	38.35	14	4.13	144	42.48
JULY *	54	672	49	7.29	457	30	487	72.47	28	4.17	564	83.93
AUGUST *	38	586	9	1.54	348	23	371	63.31	24	4.10	404	68.94
SEPTEMBER	37	667	0	0	346	63	409	61.32	114	17.09	523	78.41
OCTOBER	33	421	0	0	316	20	336	79.81	12	2.85	348	82.66
NOVEMBER	48	765	29	3.79	361	0	361	47.19	0	0.	390	50.98
DECEMBER	15	219	0	0	116	0	116	52.97	94	42.92	210	95.89
TOTAL	356	5393	187	3.47	2842	168	3010	55.81	332	6.16	3529	65.44

**partial figures*

TABLE 6. Natural control results of comparative tests using white and green paper for borer oviposition. Weighed averages of 4 tests run in November 1975 (Sao José and Barra Grande) and February 1976 (Piracicaba and Sao José).

Color of paper Strip	exposed eggs	parasitized eggs		preyed eggs		non-hatched eggs		natural control	
		No.	%	No.	%	No.	%	No.	%
white	1,617	135	8.35	1020	63.08	118	7.30	1273	78.73
green	1,770	22	1.24	1338	75.59	120	6.78	1480	83.61

TABLE 7. Natural control of borer eggs exposed in plant and ratoon cane. Records obtained from October 1975 to November 1976 in Piracicaba.

Stool age	exposed eggs	parasitized eggs		preyed eggs		non-hatched eggs		natural control	
		No.	%	No.	%	No.	%	No.	%
Plant cane	4,647	0	0.	3623	77.96	5	0.11	3628	78.07
Ratoon cane	4,689	56	1.19	3767	80.34	213	4.54	4036	86.07

Negm and Hensley. However, it was observed that some of the "non-hatched" eggs were probably damaged by sucking predators (according to newest experiences on symptoms of preyed eggs). Total preyed eggs could have been higher, especially those caused by sucking predators, than the data show. However, differences still remain when compared with results of Negm and Hensley. Results found by Sanford[4] in Louisiana are also quite different from those reported here and from those of Negm and Hensley. But, as pointed out by Sanford, his data were taken from an area that had received yearly insecticide applications for 10 or more years. Nevertheless, the high percent of parasitism (40.7%) of borer eggs found by Sanford can probably also found in Sao Paulo, if the action of predators can be eliminated. Residues of parasitized eggs chewed by predators were frequently observed. As many parasitized eggs were probably destroyed by predators, the actual rate of parasitism may have been masked.

On the other hand, previous tests in Campos, Rio de Janeiro State, showed results similar to those reported by Negm and Hensley. Two preliminary tests in April 1975, showed 37.29% average predation by chewing predators alone, and no difference was found between eggs located in the interior of cane fields (34.85%) and eggs located outside of cane fields or close to pathways (33.37%). A third test in Campos in December 1975, gave results similar to those in Sao Paulo. It showed an average 64.70% natural control, 50.22% being caused by predators, 4.52% by parasites, and 9.95% by other unknown causes. These tests also showed that natural control was higher in ratoon cane (84.62%) than in plant cane (80.56%). This difference was later confirmed in Sao Paulo (Table 7). Parasitized eggs were found in ratoon cane, while there was a complete absence of parasitized eggs in plant canes. This should be carefully studied in the future.

Differences from one location to another and from one period to another were the most striking evidence shown by these studies. These differences are probably quite decisive for future success of borer populations, and consequently one of the governing factors responsible for differential infestations found in cane fields.

In the light of the data shown in Table 6, it was concluded that white paper does not influence results of natural control. Eggs oviposited on green paper were even more preyed and parasitized than those oviposited on white paper. However, no comparisons were made between eggs on paper and eggs on cane leaves.

The major conclusion from this research is that predators appear as the major components of natural control of borer eggs. A few eggs were parasitized by *Trichogramma* sp. and another small fraction was destroyed by unknown factors (possibly adverse climate, mechanical injury, diseases, and sucking predators). These figures however, are probably masked by final action of chewing predators, which do not distinguish healthy eggs from those previously parasitized or fed on by sucking predators. These studies are being continued to discover the most important factors causing differences in the natural control of borer eggs.

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CONTROL NATURAL DE HUEVOS *DIATRAEA SACCHARALIS* (Fabr., 1794) EN CAÑAVERALES DE SAO PAULO

F. Oscar Terán

RESUMEN

Se acumularon muchos datos (desde Mayo-75 hasta Diciembre – 77) sobre sobrevivencia de huevos del barrenador en varios cañaverales comerciales, donde se indujo su control natural. Huevos recien ovipositados se colocaron en el follaje de las cañas, simulando en lo posible una situacion natural. Estas fueron inspeccionadas periodicamente hasta su completa maduracion ó destrución Los resultados muestran un control natural promedio de-76.16%, con variaciones marcadas entre las distintas localidades y años. Estas diferencias parecen decisivas para el futuro exito de las poblaciones del barrenador. Los predatores se presentan como los mayores componentes de este control natural (63.63%), mientras que los parasitos toman cuenta solo de 3.90% de los huevos, y el restante 8.63% es atribuido a otras causas (talvez clima adverso, daños mecanicos, enfermedades y hasta predatores succionadores). Sin embargo, estos datos estan probablemente en mascarados por la accion final de los predatores masticadores, quienes no distinguen huevos sanos de aquellos succionados ó parasitados.

CONTROL OF SUGARCANE SCALE INSECTS WITH FOLIAR SPRAY OF INSECTICIDE IN GUJARAT

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ABSTRACT

Insecticidal trial with foliar application of methidathion, monocrotophos, phosphamidon, dimethoate at 3 kg a.i. per hectare conducted at Navsari for three years, i.e., 1972-73, 1973-74 and 1974-75 showed that the use of phosphamidon was most effective and economical in the control of sugarcane scale insects in Gujarat.

INTRODUCTION

Sugarcane is one of the most important crops of Gujarat but is subjected to a number of pests which cause reduction in cane yield and sugar recovery. Hence, plant protection is one of the most important factors in increasing the production of sugarcane and sugar. Sugarcane scale insects, *Melanaspis glomerata* Green is one of the serious pests of sugarcane in Gujarat. Tembhekar[6] reported 30% reduction in yield as well as 35% reduction in C. C. S. per acre due to scale insects in Gujarat. Similarly Moholkar *et al*[3] reported 25% loss in weight of sugarcane in Maharashtra while Khanna[2] observed 43% loss in sugarcane and 8.03 percent loss in sucrose content due to scale insects. Weight of cane, percentage of sucrose in juice, and brix were reduced by 32.60, 44.89 and 32.98 percent, respectively, in the scale infested canes as compared to the healthy ones (Seshagiri Rao[4]). Soil application of Disulfoton granules at 3 kg. a.i./ha was found to be effective in the control of the pest (Annon[1] and Shah *et al*[5]). Seshagiri Rao[4] observed that 0.1 percent spray of malathion or dichlorvos or parathion or dimethoate or phosphamidon, twice at fortnightly intervals after detrashing the cane was effective. Thus, a study on the chemical control of this pest using new as well as available insecticides in the form of foliar sprays has been undertaken at Navsari.

MATERIALS AND METHODS

The experiment was carried out, using a Complete Randomised Block Design with four replications and five treatments including control in the Regional Sugarcane Research Station of Navsari (Lat. 20° .57′ N, Long 72° .54′ E) during the

TABLE 1. Percent incidence of scale insects and yield as well as insecticidal cost-benefit ratio in the insecticidal trial carried out with foliar applications based on results of experiments conducted for three years (1972-73, 1973-74, 1974-75).

Treatment	Average percent incidence of scale insect at harvest	Average yield ton/ha	Additional yield ton/ha	Income* Rs./ha.	Cost of Insecticide Rs./ha	Net profit after deducting cost of insecticide in comparison to control Rs/ha	Additional profit per rupee cost of insecticide (ICAR)
Dimethoate	14.97	62.500	10.782	1293.84	700.00	593.84	1 : 0.84
Phosphamidon	10.17	65.571	13.853	1662.36	450.00	1212.36	1 : 2.69
Monocrotophos	8.39	63.984	12.266	1461.92	1290.00	171.92	1 : 0.13
Methidathion	6.94	75.936	24.219	2906.28	1500.00**	1406.28	1 : 0.90
Control	21.09	51.718	–	–	–	–	–
S. E.	1.31	2.437					
C. D. @ 5%	2.85	4.940					

** Market rate of sugarcane = Rs. 120 per ton*
*** Approximate market price (personal communication)*

year 1972-73, 1973-74, and 1974-75. The variety used was sugarcane Co .419, The usual dose of fertilizer at 250 kg nitrogen, 125 kg. phosphatic acid and 125 kg potash per hectare was applied. Four insecticides, viz, dimethoate, Phosphamidon, monocrotophos and methidathion were used in the form of foliar sprays. These insecticides were applied five times at an interval of 30 days, starting on June-July with a total dose of 3 kg a.i. per hectare. Each plot size measured 10 m x 4 m.

Ten plants from each treatment were selected randomly and were tagged for observation. Percent incidence was recorded on the basis of the total number of infested internodes on each plant. Yield data obtained for 3 years were also recorded, analyzed and reported here.

RESULTS AND DISCUSSION

The data on the incidence of scale insects and yield for three years are shown in Table 1. There was minimum incidence of scale insects (6.94 percent) in plots treated with methidathion and was significantly superior among insecticidal treatments as well as control. The chronological order of effectiveness was methidathion (6.94 percent) > monocrotophos (8.39 percent) > phosphamidon (10.17 percent) > dimethoate (14.97 percent) in comparison to Control (21.09 percent). Yield data revealed that there existed significant difference for the treatments and the chronological order of effectiveness remained as methidathion > phosphamidon > monocrotophos > dimethoate in comparison to control.

Further analysis to find out Insecticidal Cost-Benefit Ratio (ICAR) to show the response of treatments in giving an additional yield (profit) per rupee cost of insecticide showed that the chronological order of effectiveness of the treatments was phosphamidon > dimethoate > methidethion > monocrotophos in comparison to control.

Thus, it can be concluded that the treatment of phosphamidon at 3 kg a.i. per hectare in the form of foliar spray proved to be best both for the reduced incidence of the pest as well as for additional yield as shown from the data in insecticidal cost-benefit ratio.

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CONTROL DE LA ESCAMA O GUAGUA DE LA CAÑA DE AZÚCAR MEDIANTE LA APLICACION FOLIAR DE INSECTICIDAS EN GUJARAT

A. H. Shah, M. S. Purokit, K. R. Patel y N. V. Vaghela

RESUMEN

Pruebas realizadas en Navsari durante los años 1972-73, 1973-74 y 1974-75 con aplicaciones foliares de monocrotophos, phosphamidon y dimethoate a razón de 3 kilogramos por hectárea cada uno separadamente, demuestran que el tratamiento con phosphamidon* es efectivo y economico para el control de insectos escamosos en caña de azucar en la region de Gujarat.

**Demecron — Dovip — Famfos*

CORRELATION BETWEEN THE INTENSITY OF INFESTATION AND POPULATION INDEX OF THE SUGARCANE BORER, *DIATRAEA SACCHARALIS (FABR., 1974).*

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ABSTRACT

Through studies of linear regression and simple correlation, an estimation of relative populations of larvae and pupae of the sugarcane borer, *Diatraea saccharalis* (Fabr., 1974) was done.

Observations were made in nine sugarmills constituting an area of approximately 179,591 ha. of sugarcane.

A high positive correlation between Intensity of Infestation and Population Index of *D. saccharalis* was evidenced. Based on Intensity of Infestation, it is possible to estimate the Population Index of the pest through simple linear regression equations.

For each year of study, the following equations were determined: $y = -3.9872 + 1.5347\ X$ ($r^2 = 0.8130$) for 1976, $Y = -0.8373 + 0.7305\ X$ ($r^2 = 0.8291$) for 1977, and $Y = -4.3104 + 1.5024\ X$ ($r^2 = 0.6417$) for 1978. Throughout the study the equation $Y = -3.7421 + 1.3712\ X$ ($r^2 = 0.6381$) was achieved in order to get a single equation which will allow the estimation of Population Index figures with minimum error. It is necessary to carry on the studies, assembling a greater amount of environment variations.

INTRODUCTION

The sugarcane borer, *Diatraea saccharalis* (Fabr., 1794) (Pyralidae, Lepidoptra) is considered the most important pest for the sugar-alcohol agro-industry of the State of Sao Paulo and population appraisal is one of the greatest problems for the sugarcane entomologists.

Except for adult populations which can be estimated through light traps (Silveira Neto[7]· Wiendl and Silveira Neto[9]; Mendes[4]) and pheromone traps (Botelho *et al*[1]), the other stages of the pest cycle present innumerable problems to be appraised.

According to Silveira Neto *et al*[8] population should be measured by applied survey methods which estimate population densities. To determine this population density, Dajoz[2] mentions several evaluation methods, assembling them in four main groups viz, direct counting of the population, methods of trapping and retrapping determination through sampling and, the indirect methods of population appraisal.

Graham and Knight[3] reported that in several cases, the relative amount of insects may be estimated through the counting of a certain number of roots, branches or stalks and concluded that the method of expressing populations in relative terms based on damages to the culture is quite satisfactory when insects are present in great numbers.

On the other hand, Morris[6] stated that the Population Indexes are frequently applied in population appraisals and that in many cases, this is the only practicable method, due to the costs involved in more accurate methods.

Yates[10] refers to costs, reporting that the best method is the one that achieves the desired accuracy at the least possible cost. Graham and Knight[3] also demonstrated that some injuries are proportional to the insect population. Thus, it is possible to express the population tendency in terms of damage.

Dajoz[2] also made reference to several authors and made trials in order to estimate the insect abundance based on damage they caused to plants. For instance, exit holes made by adults whose larvae developed inside tree trunks may be counted, or from tracks left under "olms" by beetles, the amount of individuals in various stages, from egg to adult can be determined.

Concerning sugarcane borer populations, Metcalfe[5] stated that they can be estimated through direct counting of live larvae or pupae in the field, or indirectly through signs such as empty pupae left in sugarcane stalks. The author concluded that estimations of borer population through direct methods would be harder than estimations through visible damage.

This paper attempts to determine, through studies of linear regression and simple correlation, the possibility of estimating relative populations of larvae and pupae of the sugarcane borer represented by Population Index, from data of Intensity of Infestation surveys.

MATERIALS AND METHODS

The experiment was carried out from January 1976 to December 1978,

in the following nine sugarmills in the State of Sao Paulo: Sao Joao (Araras), Tamoio (Araraquara), Costa Pinto (Piracicaba), Sao Martinho (Pradopolis), Ester (Cosmopolis), da Barra (Barra Bonita), Amalia (Sta. Rosa do Viterbo), Nova America (Assis) and Maracai (Maracaí). These constitute an area of approximately 179,591 ha. of sugarcane, i.e., 20% of the total sugarcane area of the State of Sao Paulo.

In these sugarmill areas four survey fields were delimited, based on cultivated varieties, number of cuts and types of soil. In said fields, samples of 10 stools of sugarcane were taken monthly. The total stalks, total internodes and number of externally bored internodes were counted. Samples were then dissected and all "biological forms" (4th instar and larger larvae both parasitized and unparasitized, and pupae) of the sugarcane borer were collected.

The amount of samples varied, according to the harvest period of the sugarmills. However, during the 36 months of survey, 4,623 compound samples were examined, representing a monthly average of 1,288 stools.

With these data, it was possible to calculate the Intensity of Infestation and the Population Index, using the following formulas:

$$\text{Intensity of Infestation} = \frac{\text{Bored Internodes}}{\text{Total of Internodes}} \times 100\ \%$$

$$\text{Population Index} = \frac{\text{Total of Biological Forms}}{\text{Total of Stalks}} \times 100\ \%$$

Based on these, simple correlations, between studied parameters, were established. The linear regression equation and estimation of the Population Index values with 95% probability confidence intervals (C. I.) were also determined.

From the estimated values and their confidence intervals, the curves corresponding to each year of sampling were plotted, as well as the curve for the 36 months of study.

The "t" test was applied to determine differences among the coefficients of the annual regression equations.

RESULTS AND DISCUSSION

A total of 472,882 stalks were examined, and 25,172 biological forms of the borer were found in 46,230 sampled stools, representing an average of 10.23 stalks and 0.54 biological forms per stool (Table 1).

Table 2 presents data of Intensity of Infestation (X) and values observed and estimated, through the regression equation $Y = -3.9872 + 1.5347\ X$, of the

Population Index(Y), as well as respective confidence intervals for the average monthly values of 1976. The correlation coefficient between the two parameters was highly significant ($r = 0.9017^{**}$) showing a close relationship between them. The data are shown graphically in Fig. 1.

For 1977 (Table 3), there was again a strong association ($r = 0.9106^{**}$) of the Population Index with the Intensity of Infestation. The data for 1977 are shown graphically in Fig. 2.

A high correlation coefficient ($r = 0.8011^{**}$) was also found for 1978, indicating again a strong relationship between Intensity of Infestation and the Population Index (Table 4). The data for 1978 are shown graphically in Fig. 3.

With the individual values of the 3 years, a high correlation coefficient ($r = 0.7988^{**}$) was found between the Intensity of Infestation (X) and Population Index (Y). Through the linear regression equation $Y = -3.7421 + 1.3712\ X$ the values of Y and their respective confidence intervals were estimated (Table 5)

Therefore, a positive correlation between the studied parameters was evidenced, i.e., an increase in the relative population of the pest, increases correspondingly the percentage of Intensity of Infestation. Through this, it was possible to estimate the population of larvae, from the 4th instar on, and pupae of *D. saccharalis.*

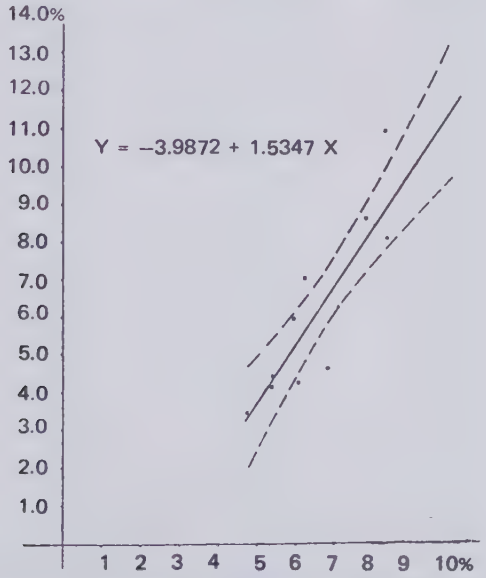

FIGURE 1. Population Indexes of *Diatraea saccharalis* (Y) and their respective confidence intervals, at the 95% level of probability, based on monthly averages of Intensity of Infestation (X), from January to December 1976.

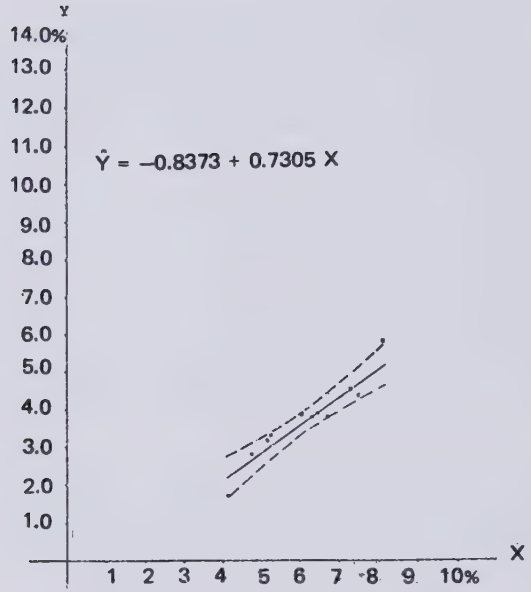

FIGURE 2. Population Indexes of *Diatraea saccharalis* (Y) and their respective confidence intervals, at the 95% level of probability, based on monthly averages of Intensity of Infestation (X), from January to December 1977.

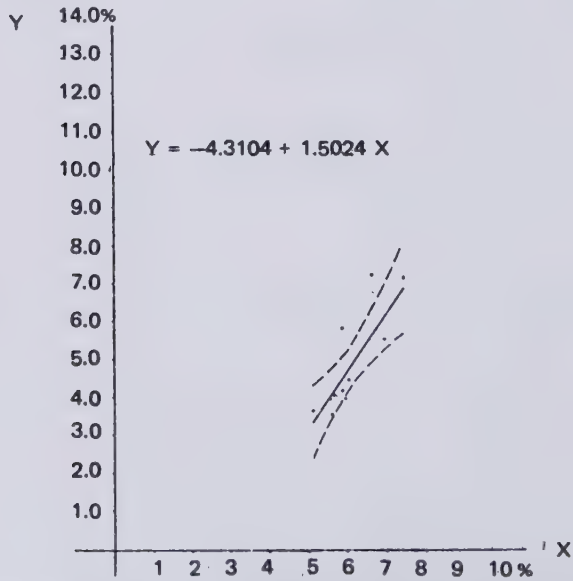

FIGURE 3. Population Indexes of *Diatraea saccharalis* (Y) and their respective confidence intervals, at the 95% level of probability, based on monthly averages of Intensity of Infestation (X), from January to December 1978.

TABLE 1. Totals of sampled stalks, internodes and bored internodes, and collected specimens of *Diatraea saccharalis,* from January 1976 to December 1978.

Month	Total of Stalks	Total of joints	Bored joints	Specimens of* Diatraea
JAN/1976	5,897	55,568	2,646	206
FEB	10,352	87,287	5,477	733
MAR	13,420	129,059	9,288	837
APR	16,109	173,579	14,552	1,304
MAY	19,335	244,480	20,405	2,115
JUN	19,349	249,190	24,922	2,101
JUL	19,361	253,767	19,749	1,668
AUG	13,298	186,675	11,180	792
SEP	8,780	126,903	7,721	372
OCT	5,758	86,771	4,675	240
NOV	3,946	51,252	2,809	174
DEC	4,698	53,489	3,657	217
JAN/1977	7,669	78,401	4,735	296
FEB	9,209	101,237	5,314	311
MAR	19,894	197,016	13,324	759
APR	21,894	237,320	17,870	960
MAY	22,488	270,795	20,025	862
JUN	22,361	288,224	18,649	870
JUL	19,991	260,278	19,146	897
AUG	11,488	155,560	12,619	667
SEP	10,143	139,207	8,772	385
OCT	8,054	99,478	5,109	256
NOV	5,961	74,168	3,093	100
DEC	8,330	86,844	4,119	236
JAN/1978	12,091	103,618	5,285	441
FEB	16,788	152,830	8,508	663
MAR	16,971	180,341	10,875	754
APR	16,770	194,302	10,973	679
MAY	21,189	260,086	14,593	1,142
JUN	19,740	249,494	14,538	1,137
JUL	15,741	202,643	13,420	745
AUG	15,275	179,125	10,653	605
SEP	9,740	127,821	8,870	512
OCT	6,745	80,558	5,602	372
NOV	7,860	83,555	4,888	328
DEC	6,097	61,004	4,508	436
TOTAL	472,882	5,561,925	372,509	25,172

(*) Specimens of Diatraea *are larvae (from 4th instar on) and pupae.*

TABLE 2. Intensity of Infestation (X) and Population Index observed (Y) and estimated (Y) with their respective confidence intervals, from January to December 1976.

X	Y (Observed)	Y (Estimated)	Confidence Interval	
			Lower Limit	Higher Limit
4.76	3.49	3.32	1.98	4.65
6.27	7.08	5.64	4.81	6.46
7.15	6.24	6.99	6.21	7.76
8.38	8.09	8.87	7.78	9.97
8.35	10.94	8.83	7.75	9.91
10.00	10.86	11.36	9.57	13.15
7.78	8.62	7.95	7.06	8.85
5.99	5.96	5.21	4.32	6.09
6.08	4.24	5.34	4.48	6.21
5.39	4.17	4.28	3.20	5.37
5.48	4.41	4.42	3.37	5.47
6.84	4.62	6.51	5.75	7.27
6.8725	6.56	6.56	5.80	7.32

$r = 0.9017^{**}$ $r^2 = 0.8130$ $F = 43.48^{**}$

$$Y = -3.9872 + 1.5347\,X$$

TABLE 3. Intensity of Infestation (X) and Population Index observed (Y) and estimated (Y), with their respective confidence intervals, from January to December 1977.

X	Y (Observed)	Y (Estimated)	Confidence Interval	
			Lower Limit	Higher Limit
6.04	3.86	3.57	3.29	3.86
5.25	3.38	3.00	2.63	3.36
6.76	3.80	4.10	3.80	4.40
7.53	4.38	4.66	4.26	5.07
7.39	3.83	4.56	4.18	4.94
6.47	3.89	3.89	3.61	4.17
7.36	4.49	4.54	4.16	4.91
8.11	5.81	5.09	4.58	5.60
6.30	3.80	3.76	3.49	4.04
5.14	3.18	2.92	2.54	3.30
4.17	1.68	2.21	1.65	2.77
4.74	2.83	2.63	2.17	3.08
6.2717	3.7442	3.7442	3.47	4.02

$r = 0.9106^{**}$ $r^2 = 0.8291$ $F = 48.52^{**}$

$$Y = -0.8373 + 0.7305\,X$$

TABLE 4. Intensity of Infestation (X) and Population Index observed (Y) and estimated (Y), with their respective confidence intervals, from January to December 1978.

X	Y (Observed)	Y (Estimated)	Confidence Interval	
			Lower Limit	Higher Limit
5.10	3.65	3.35	2.39	4.32
5.57	3.95	4.06	3.38	4.74
6.03	4.44	4.75	4.22	5.27
5.65	4.05	4.18	3.54	4.82
5.61	3.52	4.12	3.46	4.78
5.83	5.78	4.45	3.88	5.02
6.62	7.22	5.64	4.98	6.29
5.94	3.96	4.61	4.07	5.15
6.94	5.26	6.12	5.29	6.94
6.95	5.52	6.13	5.30	6.97
5.85	4.17	4.48	3.92	5.04
7.39	7.15	6.79	5.66	7.92
6.1233	4.8892	4.8892	4.37	5.41

$r = 0.8011^{**}$ $r^2 = 0.6417$ $F = 17.91^{**}$

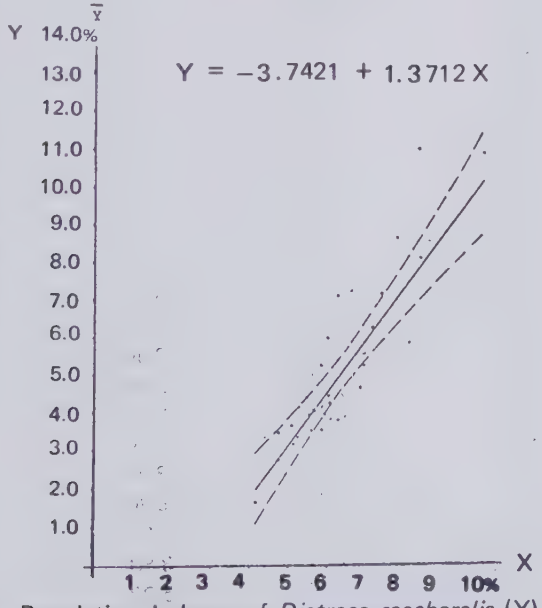

FIGURE 4. Population Indexes of *Diatraea saccharalis* (Y) and their respective confidence intervals, at the 95% level of probability, based on monthly averages of Intensity of Infestation (X), from January 1976 to December 1978.

TABLE 5. Intensity of Infestation (X) and Population Index observed (Y) and estimated (Y), with their respective confidence intervals, from January 1976 to December 1978.

X	Y (Observed)	Y (Estimated)	Confidence Interval	
			Lower Limit	Higher Limit
4.76	3.49	2.78	2.05	3.52
6.27	7.08	4.86	4.41	5.30
7.15	6.24	6.06	5.55	6.57
8.38	8.09	7.75	6.92	8.58
8.35	10.94	7.71	6.88	8.53
10.00	10.86	9.97	8.61	11.33
7.78	8.62	6.93	6.27	7.58
5.99	5.96	4.47	4.01	4.94
6.08	4.24	4.59	4.14	5.05
5.39	4.17	3.65	3.08	4.22
5.48	4.41	3.77	3.22	4.33
6.84	4.62	5.64	5.17	6.10
6.04	3.86	4.54	4.08	5.00
5.25	3.38	3.46	2.85	4.06
6.76	3.80	5.53	5.07	5.98
7.53	4.38	6.58	5.99	7.18
7.39	3.83	6.39	5.83	6.95
6.47	3.89	5.13	4.69	5.57
7.36	4.49	6.35	5.79	6.91
8.11	5.81	7.38	6.63	8.13
6.30	3.80	4.90	4.46	5.34
5.14	3.18	3.31	2.67	3.94
4.17	1.68	1.98	1.06	2.89
4.74	2.83	2.76	2.01	3.50
5.10	3.65	3.25	2.61	3.90
5.57	3.95	3.90	3.36	4.43
6.03	4.44	4.53	4.07	4.99
5.65	4.05	4.01	3.49	4.54
5.61	3.52	3.95	3.42	4.48
5.83	5.78	4.25	3.77	4.74
6.62	7.22	5.34	4.89	5.78
5.94	3.96	4.40	3.93	4.87
6.94	5.26	5.77	5.30	6.25
6.95	5.52	5.79	5.31	6.27
5.85	4.17	4.28	3.80	4.76
7.39	7.15	6.39	5.83	6.95
6.4225	5.0645	5.0644	4.63	5.50

$r = 0.7988^{**}$ $r^2 = 0.6381$ $F = 59.95^{**}$

$Y = -3.7421 + 1.3712\,X$

Fig. 4. shows the estimation of the Population Index, based on the Intensity of Infestation, through the regression equation for the period of 36 months.

With this method, it is possible to deal not only with the total population of larvae (healthy and parasitized) and pupae, but also to establish the correlations with only one of these stages.

In 1976, for instance, correlation coefficient between Intensity of Infestation and the amount of healthy borers, was determined ($r = 0.8103^{**}$). The corresponding linear regression equation was $Y = -4.0300 + 1.1179\ X$, where X represents the Intensity of Infestation and Y the estimation of the amount of healthy borers, at the time of sampling.

To check the differences among the linear regression equation during the years of study, the "t" test was applied, comparing linear and angular coefficients of the equations, resulting in the data presented in Table 6.

TABLE 6: Values of the "t" test for the contrasts established among the coefficients of regression equation of the different years.

a	1976	1977	b	1976	1977
1977	1.78 (ns)		1977	3.15*	
1978	0.12 (ns)	1.52 (ns)	1978	0.08 (ns)	2.09 (ns)

In contrast involving linear and angular coefficients of the equations of 1976, 1977 and 1978, no statistical differences among the linear coefficients were achieved. However, comparing angular coefficients for 1976 and 1977, a statistical difference at the 5% level of probability existed. This difference could be attributed to climatic factors, which were not considered in the present paper. To obtain a single equation, which will allow estimations of Population Index values with minimum error it will probably be necessary to continue the experiment in other years. This is especially evident due to the significant difference between the angular coefficients in 1976 and 1977.

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CORRELACION ENTRE LA INTENSIDAD DE INFESTACION E INDICE DE POBLACION DEL BARRENADOR DE LA CAÑA DE AZUCAR, *DIATRAEA SACCHARALIS* (FABR., 1794)

A. C. Mendes, P.S.M. Botelho, y N. Macedo

RESUMEN

En este trabajo se trata de determinar, mediante estudios de regresion linear y correlacion simple, la posibilidad de estimar poblaciones relativas de larvas y puparios del barrenador de la caña de azucar, *Diatraea saccharalis* (Fabr., 1794), representados por indices de

poblacion obtenidos por datos de intensidad de infestacion.

Entre enero de 1976 y diciembre de 1978 se hicieron los experimentos en los siguientes ingenios del estado de Sao Paulo; Sao (Joao (Araras), Tamoio (Araraquara), Costa Pinto (Piracicaba), Sao Martinho (Pradopolis), Ester (Cosmopolis), da Barra (Barra Bonita), Amália (Sta. Rosa do Viterbo), Nova América (Assis), y Maracai (Maracai). Estos 9 ingenios representan una superficie de aproximadamente 179,591 has. de caña, o sea, 20% de la superficie total en el estado de Sao Paulo.

Al terminar este experimento se llegaron a las siguientes conclusiones:

1. Fue evidente la alta correlacion entre Intensidad de Infestacion e Indice de Población.

2. Basado en el Indice de Infestacion, es posible estimar el Indice de Poblacion de la plaga mediante una ecuacion sencilla de regresion linear.

3. Durante los años de estudios se establecieron las siguientes ecuaciones:

   1976 $Y = -3.9872 + 1.5347\ X$ $(r^2 = 0.8130)$
   1977 $Y = -0.8373 + 0.7305\ X$ $(r^2 = 0.8291)$
   1978 $Y = -4.3104 + 1.5024\ X$ $(r^2 = 0.6417)$

4. En este periodo 1976-1978 se llego a la siguiente ecuacion:

   $Y = -3.7421 + 1.3712\ X$ $(r^2 = 0.6381)$

5. Para poder llegar a una sola ecuación que permita estimar el Indice de Población con un minimo de error, es necesario realizar estudios que incluyan mayor numero de variantes ambientales.

THE ECOLOGY OF WHITE GRUB OF SUGARCANE, *COCHLIOTIS MELOLONTHOIDES* GERST, WITH NOTES ON LIGHT TRAP CAPTURES OF THE ADULT BEETLES

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ABSTRACT

The white grub of sugarcane, *Cochliotis melolonthoides,* was first recorded in northern region of Tanzania by Jepson[1]. Although the white grub was then regarded as a minor pest of sugarcane in northern Tanzania it has, for the last several years, caused heavy losses. Light trap catches of the adult beetles in this study indicated that the pest has two swarming periods each year occurring during the long and short rain periods in March to May, and September to December, respectively. This species has three larval instars but it was observed that the generation always overlapped.

INTRODUCTION

The white grub of sugarcane, *Cochliotis melolonthoides,* (Coleoptera: Melolonthoides) was first recorded in the slopes of Mt. Kilimanjaro in the northern region of Tanzania by Jepson[1]. He found *Compsomeris mansueta, C. felina, C. lachesis,* and *C. caelebs* to be effective hymenopteran parasites. He also found elaterid larvae to be predating on white grubs. Passalid larvae of *Didimus sansibaricus* were also found to be predating on *Cochliotis* and were common locally. About 0.5% of *Cochliotis* larvae dissected were found to contain an unidentified nematode.

Six species of chafer beetles have been recorded at the Tanganyika Planting Sugarcane Company at Moshi in the northern region of Tanzania. These in order of their relative importance were (1) *Cochliotis melolonthoides* (Gerst), *Anomala exitalis* (Per), *Heteronychus tunuestriatus* (Feirm), *Anomala* (Gerst), *Entyposis impressa* (Kolbe), and *Adoretus versutus* (Har).

The most serious pest, *C. melolonthoides,* has three larval instars with the third instar causing the greatest damage. These larvae are generally found immediately beneath cane stools in infested fields. Normally, only cane roots are eaten by the grubs although in some cases the base of the cane stalk is also eaten and larvae may tunnel into it. Infested cane shows signs of water

stress and lodging occurs in severely infested cane, and infested cane may deteriorate to such a degree that harvesting becomes uneconomic.

C. melolonthoides in northern Tanzania had been observed to reduce yield from 125 tons/ha of plant cane and 60 tons/ha of ratoon crop, to an average of 50 tons/ha in both plant cane and ratoon crop.

BIOLOGICAL OBSERVATIONS

The life cycle of the white grub was observed in this study to follow a general pattern. Adult beetle have two swarming periods. The first and smaller swarming period was observed from the end of March until the end of May, with a peak in April. The second, and larger swarming period began after the middle of September and continued until early December with a peak occurring in October.

Before the adults emerged at about 18.30 hours during swarming they emitted a whizzing sound. The sound was immediately followed by an almost simultaneous emergence of thousands of adult beetles. Copulation took place during swarming although a few adults were observed to emerge from the soil in copula.

Swarming was always observed to take place around eucalyptus trees and lasted for about twenty minutes. Afterwards all the adult beetles burrowed in the soil. Females were observed to burrow a considerable depth in the soil before ovipositing after swarming. This depended on the soil texture and moisture. In hard dry soil the eggs were found only about 30 cm. below soil surface, whereas in fine moist soil they were found as deep as one meter below the soil surface. The variable depth of oviposition might explain the overlapping of generations observed in the field. The eggs near the soil surface where temperature was much higher probably hatched earlier, and the larvae developed faster.

Although the larvae fed on sugarcane roots, in this study larvae were also maintained in the laboratory for three weeks in petri dishes without food.

Larval population density studies were conducted at the sugarcane estate by digging out soil samples. Each sample pit measured 1.0 meter by 0.5 meter wide, and 0.25 meter deep. Taplay[2] examined 18 pits over 2 hectares, while Jepson[1] examined 10 pits and concluded that 2,000 to 16,000 grubs per hectare constituted a light infestation, 16,000 to 40,000 grubs/ha as medium infestation, and over 40,000 grubs/ha heavy infestation. In this study, after examining an average of 50 pits/ha every year for a period of 2 years, grub population per hectare was estimated to be between 200,000 and 300,000 grubs per hectare.

Pupation was observed to take place about 10 cm. below the soil surface.

TABLE 1. Light trap catches of *Cochliotis melolonthoides* adult beetles (together with rainfall in mm) for the period June 1967 to June 1970.

Month	1967		1968		1969		1970	
	Adults	Rainfall	Adults	Rainfall	Adults	Rainfall	Adults	Rainfall
January	–	2.40	–	6.70	–	21.10	–	
February	–	7.20	6	50.30	–	20.10	1	
March	–	39.10	172	158.00	154	97.60	416	
April	–	80.10	17	419.30	70	45.30	271	
May	–	82.50	–	121.10	–	18.30	–	
June	–	11.20	–	28.30	–	0.50	–	
July	–	31.20	–	3.90	–	0.00	–	
August	–	13.00	–	20.20	–	14.80	–	
September	–	45.90	27	4.00	18	3.20	–	
October	215	17.80	318	14.50	434	35.00	–	
November	109	39.80	117	126.30	72	87.60	–	
December	1	29.30	3	110.10	–	7.30	–	
Total	325	399.50 (mm)	660	1062.70 (mm)	748	350.80	688	

LIGHT TRAPPING OF ADULT BEETLES

Adult beetles were trapped using a Robinson-type light trap operated between 18.00 hours and 6.30 hours. The trap was fitted with MB/U, 200/250 volt mercury vapor bulb, and tetrachlorothane was used as a killing agent.

The results of the total monthly capture of the adult beetles from June 1967 to June 1970 are shown in Table 1. From this table the two clearly defined peaks were evident each year. These peaks were directly associated with periods of high rainfall.

CONTROL METHODS

In the course of this study, birds (storks) were found to be good predators of the grubs. The storks were only found following harrowing after cane harvesting. When the tractors worked later in the same but grub-free fields during levelling or hilling, not one single stork was observed.

The only grub parasite observed in the course of this study was *Cordyceps*. This fungus was found widely spread at the estate, especially along the moist irrigation canals. In contrast to the finding of Jepson[1] high population of elaterid beetle larvae were found to reduce germination by attacking the buds of planting material, but there was no evidence that these elaterids were predating on white grubs.

DISCUSSION

White grub infestation showed variations among different fields and also within individual fields. Infestation was observed to be much heavier in poorly developed canes.

Observations tend to indicate that adult females preferred open young cane fields rather than the fields with mature cane for egg oviposition. From the light trap data it seems that two populations of *C. melolonthoides* do exist simultaneously: those emerging during the long rains (March-April) and those emerging during the short rains (October-November). Larval samples taken at the end of September 1968, 1969, 1970 and 1971 showed that over 90% of the existing larval population were first larval instars. These were obviously the first instar larvae from adults swarming in September.

Similar sampling in December showed that 60% of the populations were third and final instars. The remaining 40% being a mixture of first and second larval instars. Third larval instars must have been derived from March-April swarming adults, while first and second larval instars must have been derived from October-November swarming adults. It would also appear that March-April larval population suffered heavy-mortality during the cold, wet periods of March, April, May, June and July.

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LA ECOLOGIA DEL GUSANO BLANCO DE LA CAÑA DE AZUCAR *COCHLIOTIS MELOLONTHOIDAS* GERST, CON NOTAS SOBRE LOS ESCARABAJOS ADULTAS ATRAPADOS.

J. N. Waiyaki

RESUMEN

La primera constancia de la presencia del Gusano Banco* de la caña de azucar en la zona norte de Tanzania, fue aportada por Jepson[1]. A pesar del insecto que esta considerado como una plaga de menor importancia en el norte de Tanzania, ha llegado a ocasionar grandes perdidas durante estos ultimos años. Escarabajos** adultos atrapados en este estudio mediante trampas con luces, indican que esta plaga tiene dos ciclos de enjambres cada año. Suceden durante los periodos lluviosos de larga y corta duracion, de marzo a mayo y de septiembre a diciembre respectivamente. Las larvas pasan por tres mudas, aunque las generaciones siempre se traslapan.

* *Gusano de manteca, gallina ciega, nixticuil, caculo, quetepereque, pao-de-galinha.*

** *Mayate, chicharron, gallego, besouro da caña.*

ASPECTS OF THE POPULATION DYNAMICS OF *APANTELES FLAVIPES* (CAMERON) AND SUPPORT CAPACITY OF ITS HOST *DIATRAEA SACCHARALIS* (FABR.)

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ABSTRACT

This work aimed at measuring under field conditions, the dispersion capacity and the interaction model between the parasite *Apanteles flavipes* and the host *Diatraea saccharalis* as well as the support capacity of this host under laboratory conditions.

The results allowed the following conclusions:

a) The average dispersion capacity of *A. flavipes* was 34.38 meters;

b) The area of discovery of the parasite *A. flavipes*, according to Nicholson's model, was 0.031 m^2 ;

c) The population of searching parasites in the following generation, resulting from the release of approximately 24,600 *A. flavipes* females was 1,838 cocoons in an area of 1.13 ha;

d) The average mortality of *A. flavipes* adults directly increased in relation to the instar of the host *D. saccharalis*; and,

e) The borer presented a support capacity to *A. flavipes* parasitism from the 3rd instar on, being superior in the 5th and 6th instars.

INTRODUCTION

According to Moutia and Courtois[11], *Apanteles flavipes* was originally described by Cameron in 1891 in Poona, India. It was redescribed by Wilkinson in 1928. It is geographically distributed in India, Ceylon, Formosa, Japan, Australia, Malaysia and Mauritius.

This insect is a primary larval parasite of pyralids and noctuids. According to Gifford and Mann[7], it seems to be as proliferous on *Diatraea saccharalis* as on its original hosts. Its adaptation has been successful in Mauritius since 1952, in Reunion since 1964, in Antilles and Barbados since 1967 and in Saint Kitts since 1970 (Delattre[3]). The adaptation of this parasite has also been confirmed in Colombia (Gaviria[5,6]) and in Guyana (Des Vignes[4]).

In Brazil, the first positive results concerning the adaptation of *A. flavipes* were achieved in Alagoas. After 32 months of its introduction in the state (PLANALSUCAR[12]) the average parasitism by *A. flavipes* reached 8.38%, which represents 46.24% of the total parasitism in Alagoas. After a rapid adaptation in the state of Bahia, the average parasitism reached 10.97%, which is quite close to the one reached by the most effective parasite up to that time, *Metagonistylum minense* (Towns., 1927).

In spite of the success of the *A. flavipes* to control *D. saccharalis,* in the Northeast of Brazil its adaptation to the state of Sao Paulo conditions has been slower. This difficulty has been overcome by mass rearing of the insect by PLANALSUCAR laboratory or by the laboratories in Sugarmills, under the assistance of PLANALSUCAR technicians. In 1978, these laboratories produced and released approximately 50 million *A. flavipes*.

Due to the natural difficulties in establishing a biological control program in the Central-South region of Brazil, this program provided basic information concerning the parasite behavior following mass production and liberation, thus granting better chances of success.

This work was undertaken to determine, under field conditions in the region of Araras, state of Sao Paulo, the average dispersion of *A. flavipes* and the interaction model between this parasite and its host. Also evaluated was the rate of parasitism by *A. flavipes* supported by *D. saccharalis*.

MATERIAL AND METHOD

A. flavipes *dispersion*

This trial was carried out at Sao Joao Sugarmill, Araras, state of Sao Paulo.

The parasites were supplied by the Laboratory of Biological Control of the Southern Regional Co-ordination Service of PLANALSUCAR. Many of the 1,230 cocoons were kept in laboratory until adult emergence, after which they were transported and released in the fields.

From November 1978 to April 1979, 5 releases took place in previously selected blocks planted with the sugarcane variety NA 56-79 (plant cane). These blocks were far from one another, and *A. flavipes* did not occur naturally in these areas.

In each block, approximately 44,280 adults of *A. flavipes* were released. The sexual ratio of 1 male for 1.27 females resulted from the release of about 24,600 females.

A fortnight after releases, the biological material (hosts and live parasites) were collected, in circles, at the distances of 0-20, 20-40, 40-60, 60-80, 80-100, 100-120 and 120-140 meters from the release area. In each distance, in 8 places randomly chosen, 15 "biological forms" (larvae and pupae) were collected, totaling 120 biological forms per circle.

The collected material was brought to the laboratory, where it was separated and recorded. The live larvae were kept in plastic boxes, and fed with sugarcane bits, in order to make a selection of the healthy from the parasitized material possible. After 15 days, this material was reviewed, and eliminated after the data were recorded.

Finally, with the data from field surveys (*A. flavipes* cocoons recovered), the dispersion was calculated. This value was derived from a determined mean, where the number of recovered cocoons and the distances within which they were found were considered. The final value was achieved through the average of treatment means.

Area of discovery of the parasite A. flavipes *and expected population*

In order to obtain the data for this experiment, samples were taken from the same localities of the three last replications of the previous experiment.

A fortnight after the release of the parasites, 8 random samples were taken at the following distances: 0-20, 20-40, and 40-60 meters, from the area of release. In each of these circles, 120 stalks were taken (15 stalks per sample spot). From these all biological materials such as larvae and pupae, were removed. Near each sample area, the number of stalks per linear meter were counted (8 replications per circle) for an estimation of the average number of stalks per hectare.

Knowing the approximate number of stalks per hectare and the number of biological forms found in the 120 stalks examined per circle, it was possible to estimate the number (or density) of hosts subject to the attack of the parasite (N) and the number (or density) of non-parasitized hosts (S). To calculate the parasite area of discovery (A), the following Nicholson's mathematic model was used (Varley *et al*[13]):

$$a = \frac{1}{p} \log_e \frac{N}{S}$$

where p is the number of released parasites (female).

Knowing the parasite area of discovery, the following equation was applied:

$$P_n + 1 = N_n - \text{antilog}\left(\log N_n - \frac{ap_n}{2.3}\right)$$

where p_n and p_{n+1} are the successive parasite populations and N_n, the host population. The equation allows the estimation of the amount of searching parasites in the following generation.

Parasitism support by D. saccharalis *larvae*

Together with field trials, a trial under laboratory conditions (temperature of 27°C ±2) was also carried out to check the parasitism support of *D. saccharalis* to the attack of *A. flavipes.*

This experiment was made with 20 larvae from the 3rd to the 6th instar (totaling 80 larvae), reared on the slightly modified Hensley and Hammond's[9] artificial diet. Each lot of 80 larvae was considered a plot, and the experiment was replicated 10 times.

The larvae stung by the parasite were kept in plastic boxed with diet for feeding. This material was examined every day and the cocoons of *A. flavipes* collected. These cocoons were kept in plastic boxes for later observation on the percentage of efficiency $\left(\frac{\text{amount of cocoons} \times 100}{\text{total of stung larvae}}\right)$, number of days required for emergence of adults, and mean longevity of adults in days. The number of cocoons in each mass and the number of adults which have emerged was also determined.

In order to check the difference among the various instar, the variance analysis was used and the test of Tukey applied.

RESULTS AND DISCUSSIONS

A. flavipes *dispersion*

The data used for the dispersion calculation and the values achieved are shown in Table 1.

According to these results, it can be observed that the average of dispersion varied from 25.33 to 48.12 meters, resulting in an overall average of 34.38 meters.

Although this dispersion was small, embracing an area of approximately 0.4 ha, it can be considered an advantage in case of *A. flavipes.* Since the parasite has been introduced in the region and this small dispersion promoted the occurrence of mating, the presence of females in the subsequent generation was ensured because this insect presented a facultative haploid arrhenotokous parthe-

nogenesis. Therefore, these dispersion areas in the field could constitute initial spots

TABLE 1. Cocoons of *Apanteles flavipes* (Cameron) recovered in the field, per distance, replication, total and weighted mean (Araras, SP).

Distance in meters	Replications					TOTAL
	1	2	3	4	5	
0 – 20	9	18	15	16	11	69
20 – 40	4	4	8	6	4	26
40 – 60	1	4	5	3	0	13
60 – 80	0	0	1	1	0	2
80 – 100	0	0	0	2	0	2
100 – 120	0	0	1	2	0	3
120 – 140	0	0	1	2	0	3
Weighted Means	28.57	29.23	40.64	48.12	25.33	34.38

of distribution of the parasite, ensuring its adaptation in the region. On the other hand, if this parasite showed a high dispersion, it is most probable that after some generations the population would disappear from this area due to the lack of opportunity for mating. This information agreed with the work of DeBach and Bartlett[2] who considered this as an important factor for the introduction of a parasite in any area.

Based on the dispersion achieved the release of this insect shall be recommended to be made in locations approximately 70 meters apart in borer infested sugarcane areas.

Area of discovery of the parasite **A. flavipes** *and expected population.*

In order to measure the area of discovery of the parasite *A. flavipes,* the biological forms were sampled in the field, and the data were shown in Table 2.

To apply the Nicholson's formula, $a = \frac{I}{p} \log_e \frac{N}{S}$, the values of P, N and S were determined.

The determination of the value of P was based on data of Table 3, where it was determined that the average of adults resulting from cocoons was approximately 36 (35.94) individuals. Considering the sexual ratio 1:1.27, the result showed 20 females per cocoon. A total of 1,230 cocoons had been released, thus about 24,600 *A. flavipes* females were released (P).

To determine the values of N and S, the number of stalks per meter, was initially calculated. An estimated 145,379 stalks were in an area of approximately 1.13 ha. with a 60 meter radius.

One hundred eighty seven biological forms were present in 1,080 stalks examined (Table 2) while 25,172 hosts in 145,379 stalks were exposed to the attack of the parasite (N). If the same procedure was applied to figures in Table 2, it was possible to determine the number of non-parasited hosts (S), which amounted to 23,288 individuals.

TABLE 2. "Biological forms" examined in 120 stalks per distance, replication and total (Araras, SP).

Biological Material	Distances in meters and Replications									General
	0 – 20			20 – 40			40 – 60			
	1ª	2ª	3ª	1ª	2ª	3ª	1ª	2ª	3ª	Total
Healthy borers	14	11	21	15	18	21	19	16	15	150
live pup. *M. minense*	2	0	2	0	4	3	1	2	3	17
live pup. *P. claripalpis*	0	1	2	0	0	1	0	0	0	4
live cocoons *A. flavipes*	3	3	1	2	0	0	4	1	0	14
live pup. *Ipobracon* sp.	1	0	0	0	0	0	0	0	0	1
Borers with *Beauveria* sp.	0	0	1	1	0	0	0	0	1	3

Applying the Nicholson's formula, the value achieved was 0.031 m^2 (a), corresponding to the area of discovery of the parasite.

The next step was the calculation of the number of searching parasites in the following generation, which resulted in 1,838 cocoons ($P_n + 1$).

These results were achieved according to Nicholson's mathematic model, which supposes that there is no limitation in the oviposition, that the search is randomized and that the area of discovery is constant. However, since it is a new parasite still being introduced in the region, the amount of information concerning its behavior under field conditions is small, and perhaps a more detailed study on some aspects that may interfere in the parasite population such as host density, handling time (interval between the first host-parasite encounter and search being resumed – Holling[10]), density of the parasite, constant of interference (Hassel and Varley[8]) and possibly others, would better characterize the interaction model of parasite and host.

However, by the number of cocoons recovered in the field (Table 2), the

TABLE 3. Average number of cocoons, adults, average survival in days and % of efficiency of *Apanteles flavipes* (Cameron) achieved through inoculations of parasite on *Diatraea saccharalis* (Fabr.) larvae from the 3rd to the 6th instars. Statistical analysis (Araras, SP).

Instars	Replications Variants	1	2	3	4	5	6	7	8	9	10	MEAN	F	TUKEY (+) 5%
3º		30.60	31.71	46.10	43.86	36.10	42.38	41.33	44.00	37.14	50.16	40.34		
4º	Cocoons	34.00	33.75	36.27	40.80	26.13	46.25	34.83	45.29	47.87	39.43	38.46	ns	
5º		45.43	44.27	37.54	35.13	49.50	42.50	51.36	42.29	51.19	33.07	43.23		—
6º		36.19	34.45	33.87	35.47	39.57	45.00	45.29	40.58	53.07	39.67	40.32		
3º		27.80	28.43	44.20	38.86	32.00	38.50	38.00	41.00	33.14	47.66	36.96		
4º	Adults	28.75	29.13	27.82	34.90	21.00	41.81	32.17	39.57	45.20	37.00	33.74	ns	–
5º		38.14	40.07	27.00	29.56	46.58	36.30	43.36	26.86	45.88	25.67	35.94		
6º		28.94	29.36	25.07	24.73	33.14	37.82	36.64	28.58	50.14	30.87	32.53		
3º		2.10	2.95	2.78	2.56	2.03	2.39	2.89	2.04	3.31	2.11	2.52		b
4º	Survival	2.14	1.99	2.46	2.23	1.96	2.16	2.19	2.27	2.15	2.47	2.20	6.51**	ab
5º		1.97	1.73	2.25	2.17	1.77	2.28	2.34	1.89	1.94	2.31	2.06		a
6º		1.90	1.84	2.22	2.02	1.62	2.02	1.85	2.03	2.38	2.03	1.99		a
3º		25.00	35.00	50.00	35.00	50.00	40.00	45.00	10.00	35.00	30.00	35.00		a
4º	% Efficiency	40.00	40.00	55.00	50.00	40.00	80.00	30.00	35.00	75.00	35.00	48.00	19.94**	a
5º		70.00	75.00	65.00	80.00	60.00	50.00	70.00	70.00	80.00	75.00	69.50		b
6º		80.00	55.00	75.00	75.00	70.00	85.00	70.00	60.00	70.00	75.00	71.50		b

(+) The means followed by different letters are statistically different.

value of 1,885 cocoons of *A. flavipes* in the studied area (1.13 ha) can be calculated and this value was almost the same as the one achieved by the formula of Nicholson's model in 1938. It suggested that the parasite should present a behavior similar to the Chalcid parasite *Nasonia vitripennis* (Wirk), which according to DeBach and Smith[1], followed the same model.

Parasitism support by **D. saccharalis** *larvae*

Laboratory studies were conducted to determine the support capacity of *D. saccharalis* to the attack of *A. flavipes*, (data is shown in Table 3).

There were no significant differences between host instars studied in the production of parasite cocoons and adults, indicating a similar behaviour in any of these instars. However, when the longevity and percentage of parasitism were determined, some differences became evident. The mean longevity was shorter in individuals coming from the 5th and 6th instars, possibly due to the quality of food available for the parasite in different instars of the host. However this point deserves further investigation. The percent efficiency of the parasite was higher in host larvae of the 5th and 6th instars, indicating a better capacity of support of the host to the parasitism. This led us, inspite of the shorter longevity, to work in the laboratory with hosts of the 5th and 6th instars for the mass production of the parasite *A. flavipes.*

These results showed that in the field, *D. saccharalis* was susceptible to the parasitism by *A. flavipes* from the 3rd instar on.

CONCLUSIONS

The results of this work allowed the following conclusions:

a) The average dispersion of *A. flavipes* was 34.39 meters;

b) The area of discovery of the parasite *A. flavipes,* according to Nicholson's model was 0.031 m^2;

c) The population of searching parasites in the following generation, resulting from the release of approximately 24,600 *A. flavipes* females was 1,838 cocoons in an area of 1.13 ha;

d) The average longevity of *A. flavipes* adults decreased in relation to the instars of the host *D. saccharalis;*

e) The borer presented a support capacity to *A. flavipes* parasitism from the 3rd instar on, being superior in the 5th and 6th instars.

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ASPECTOS DE LA DINAMICA DE POBLACIONES DE *APANTELES FLAVIPES* (CAMERON) Y LA CAPACIDAD DE SUBSISTENCIA DE SU HOSPEDERO, *DIATRAEA SACCHARALIS* (FABR.)

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RESUMEN

El objetivo de este trabajo es el de calcular en condiciones de campo, la capacidad de dispersion y el patron de interaccion o comportamiento entre el parasito, *Apanteles flavipes* (Cameron, 1891) y el hospedero, *Diatraea saccharalis* (Fabr., 1794). Tambien conocer el poder de subsistencia de este hospedero en condiciones de laboratorio.

Los resultados obtenidos nos permiten llegar a las siguientes conclusiones:

a) La capacidad de dispersion promedio de *A. flavipes* fue de 34.38 metros.

b) El area de hallazgo del parasito *A. flavipes*, de acuerdo con el patron de Nicholson, fue de 0.031 m^2.

c) La poblacion de parasitos activos en la siguiente generacion, despues de haber liberado aproximadamente 24,600 hembras de *A. flavipes*, fue de 1838 capullos en una superficie de 1.13 has.

d) La mortalidad promedio de adultos de *A. flavipes* aumento con relacion al estadio de hospedero, *D. saccharalis*.

e) El barrenador tuvo un poder de subsistencia al parasitismo desde el tercer estadio, siendo superior en el quinto y sexto estadio.

poblacion obtenidos por datos de intensidad de infestacion.

Entre enero de 1976 y diciembre de 1978 se hicieron los experimentos en los siguientes ingenios del estado de Sao Paulo; Sao (Joao (Araras), Tamoio (Araraquara), Costa Pinto (Piracicaba), Sao Martinho (Pradopolis), Ester (Cosmopolis), da Barra (Barra Bonita), Amália (Sta. Rosa do Viterbo), Nova América (Assis), y Maracai (Maracai). Estos 9 ingenios representan una superficie de aproximadamente 179,591 has. de caña, o sea, 20% de la superficie total en el estado de Sao Paulo.

Al terminar este experimento se llegaron a las siguientes conclusiones:

1. Fue evidente la alta correlacion entre Intensidad de Infestacion e Indice de Población.

2. Basado en el Indice de Infestacion, es posible estimar el Indice de Poblacion de la plaga mediante una ecuacion sencilla de regresion linear.

3. Durante los años de estudios se establecieron las siguientes ecuaciones:

   1976 $Y = -3.9872 + 1.5347\ X$ $(r^2 = 0.8130)$
   1977 $Y = -0.8373 + 0.7305\ X$ $(r^2 = 0.8291)$
   1978 $Y = -4.3104 + 1.5024\ X$ $(r^2 = 0.6417)$

4. En este periodo 1976-1978 se llego a la siguiente ecuacion:

   $Y = -3.7421 + 1.3712\ X$ $(r^2 = 0.6381)$

5. Para poder llegar a una sola ecuación que permita estimar el Indice de Población con un minimo de error, es necesario realizar estudios que incluyan mayor numero de variantes ambientales.

THE ECOLOGY OF WHITE GRUB OF SUGARCANE, *COCHLIOTIS MELOLONTHOIDES* GERST, WITH NOTES ON LIGHT TRAP CAPTURES OF THE ADULT BEETLES

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ABSTRACT

The white grub of sugarcane, *Cochliotis melolonthoides,* was first recorded in northern region of Tanzania by Jepson[1]. Although the white grub was then regarded as a minor pest of sugarcane in northern Tanzania it has, for the last several years, caused heavy losses. Light trap catches of the adult beetles in this study indicated that the pest has two swarming periods each year occurring during the long and short rain periods in March to May, and September to December, respectively. This species has three larval instars but it was observed that the generation always overlapped.

INTRODUCTION

The white grub of sugarcane, *Cochliotis melolonthoides,* (Coleoptera: Melolonthoides) was first recorded in the slopes of Mt. Kilimanjaro in the northern region of Tanzania by Jepson[1]. He found *Compsomeris mansueta, C. felina, C. lachesis,* and *C. caelebs* to be effective hymenopteran parasites. He also found elaterid larvae to be predating on white grubs. Passalid larvae of *Didimus sansibaricus* were also found to be predating on *Cochliotis* and were common locally. About 0.5% of *Cochliotis* larvae dissected were found to contain an unidentified nematode.

Six species of chafer beetles have been recorded at the Tanganyika Planting Sugarcane Company at Moshi in the northern region of Tanzania. These in order of their relative importance were (1) *Cochliotis melolonthoides* (Gerst), *Anomala exitalis* (Per), *Heteronychus tunuestriatus* (Feirm), *Anomala* (Gerst), *Entyposis impressa* (Kolbe), and *Adoretus versutus* (Har).

The most serious pest, *C. melolonthoides,* has three larval instars with the third instar causing the greatest damage. These larvae are generally found immediately beneath cane stools in infested fields. Normally, only cane roots are eaten by the grubs although in some cases the base of the cane stalk is also eaten and larvae may tunnel into it. Infested cane shows signs of water

stress and lodging occurs in severely infested cane, and infested cane may deteriorate to such a degree that harvesting becomes uneconomic.

C. melolonthoides in northern Tanzania had been observed to reduce yield from 125 tons/ha of plant cane and 60 tons/ha of ratoon crop, to an average of 50 tons/ha in both plant cane and ratoon crop.

BIOLOGICAL OBSERVATIONS

The life cycle of the white grub was observed in this study to follow a general pattern. Adult beetle have two swarming periods. The first and smaller swarming period was observed from the end of March until the end of May, with a peak in April. The second, and larger swarming period began after the middle of September and continued until early December with a peak occurring in October.

Before the adults emerged at about 18.30 hours during swarming they emitted a whizzing sound. The sound was immediately followed by an almost simultaneous emergence of thousands of adult beetles. Copulation took place during swarming although a few adults were observed to emerge from the soil in copula.

Swarming was always observed to take place around eucalyptus trees and lasted for about twenty minutes. Afterwards all the adult beetles burrowed in the soil. Females were observed to burrow a considerable depth in the soil before ovipositing after swarming. This depended on the soil texture and moisture. In hard dry soil the eggs were found only about 30 cm. below soil surface, whereas in fine moist soil they were found as deep as one meter below the soil surface. The variable depth of oviposition might explain the overlapping of generations observed in the field. The eggs near the soil surface where temperature was much higher probably hatched earlier, and the larvae developed faster.

Although the larvae fed on sugarcane roots, in this study larvae were also maintained in the laboratory for three weeks in petri dishes without food.

Larval population density studies were conducted at the sugarcane estate by digging out soil samples. Each sample pit measured 1.0 meter by 0.5 meter wide, and 0.25 meter deep. Taplay[2] examined 18 pits over 2 hectares, while Jepson[1] examined 10 pits and concluded that 2,000 to 16,000 grubs per hectare constituted a light infestation, 16,000 to 40,000 grubs/ha as medium infestation, and over 40,000 grubs/ha heavy infestation. In this study, after examining an average of 50 pits/ha every year for a period of 2 years, grub population per hectare was estimated to be between 200,000 and 300,000 grubs per hectare.

Pupation was observed to take place about 10 cm. below the soil surface.

TABLE 1. Light trap catches of *Cochliotis melolonthoides* adult beetles (together with rainfall in mm) for the period June 1967 to June 1970.

Month	1967		1968		1969		1970	
	Adults	Rainfall	Adults	Rainfall	Adults	Rainfall	Adults	Rainfall
January	–	2.40	–	6.70	–	21.10	–	
February	–	7.20	6	50.30	–	20.10	1	
March	–	39.10	172	158.00	154	97.60	416	
April	–	80.10	17	419.30	70	45.30	271	
May	–	82.50	–	121.10	–	18.30	–	
June	–	11.20	–	28.30	–	0.50	–	
July	–	31.20	–	3.90	–	0.00	–	
August	–	13.00	–	20.20	–	14.80	–	
September	–	45.90	27	4.00	18	3.20	–	
October	215	17.80	318	14.50	434	35.00	–	
November	109	39.80	117	126.30	72	87.60	–	
December	1	29.30	3	110.10	–	7.30	–	
Total	325	399.50 (mm)	660	1062.70 (mm)	748	350.80	688	

LIGHT TRAPPING OF ADULT BEETLES

Adult beetles were trapped using a Robinson-type light trap operated between 18.00 hours and 6.30 hours. The trap was fitted with MB/U, 200/250 volt mercury vapor bulb, and tetrachlorothane was used as a killing agent.

The results of the total monthly capture of the adult beetles from June 1967 to June 1970 are shown in Table 1. From this table the two clearly defined peaks were evident each year. These peaks were directly associated with periods of high rainfall.

CONTROL METHODS

In the course of this study, birds (storks) were found to be good predators of the grubs. The storks were only found following harrowing after cane harvesting. When the tractors worked later in the same but grub-free fields during levelling or hilling, not one single stork was observed.

The only grub parasite observed in the course of this study was *Cordyceps*. This fungus was found widely spread at the estate, especially along the moist irrigation canals. In contrast to the finding of Jepson[1] high population of elaterid beetle larvae were found to reduce germination by attacking the buds of planting material, but there was no evidence that these elaterids were predating on white grubs.

DISCUSSION

White grub infestation showed variations among different fields and also within individual fields. Infestation was observed to be much heavier in poorly developed canes.

Observations tend to indicate that adult females preferred open young cane fields rather than the fields with mature cane for egg oviposition. From the light trap data it seems that two populations of *C. melolonthoides* do exist simultaneously: those emerging during the long rains (March-April) and those emerging during the short rains (October-November). Larval samples taken at the end of September 1968, 1969, 1970 and 1971 showed that over 90% of the existing larval population were first larval instars. These were obviously the first instar larvae from adults swarming in September.

Similar sampling in December showed that 60% of the populations were third and final instars. The remaining 40% being a mixture of first and second larval instars. Third larval instars must have been derived from March-April swarming adults, while first and second larval instars must have been derived from October-November swarming adults. It would also appear that March-April larval population suffered heavy-mortality during the cold, wet periods of March, April, May, June and July.

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LA ECOLOGIA DEL GUSANO BLANCO DE LA CAÑA DE AZUCAR *COCHLIOTIS MELOLONTHOIDAS* GERST, CON NOTAS SOBRE LOS ESCARABAJOS ADULTAS ATRAPADOS.

J. N. Waiyaki

RESUMEN

La primera constancia de la presencia del Gusano Banco* de la caña de azucar en la zona norte de Tanzania, fue aportada por Jepson[1]. A pesar del insecto que esta considerado como una plaga de menor importancia en el norte de Tanzania, ha llegado a ocasionar grandes perdidas durante estos ultimos años. Escarabajos** adultos atrapados en este estudio mediante trampas con luces, indican que esta plaga tiene dos ciclos de enjambres cada año. Suceden durante los periodos lluviosos de larga y corta duracion, de marzo a mayo y de septiembre a diciembre respectivamente. Las larvas pasan por tres mudas, aunque las generaciones siempre se traslapan.

* *Gusano de manteca, gallina ciega, nixticuil, caculo, quetepereque, pao-de-galinha.*

** *Mayate, chicharron, gallego, besouro da caña.*

ASPECTS OF THE POPULATION DYNAMICS OF *APANTELES FLAVIPES* (CAMERON) AND SUPPORT CAPACITY OF ITS HOST *DIATRAEA SACCHARALIS* (FABR.)

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ABSTRACT

This work aimed at measuring under field conditions, the dispersion capacity and the interaction model between the parasite *Apanteles flavipes* and the host *Diatraea saccharalis* as well as the support capacity of this host under laboratory conditions.

The results allowed the following conclusions:

a) The average dispersion capacity of *A. flavipes* was 34.38 meters;

b) The area of discovery of the parasite *A. flavipes*, according to Nicholson's model, was 0.031 m^2;

c) The population of searching parasites in the following generation, resulting from the release of approximately 24,600 *A. flavipes* females was 1,838 cocoons in an area of 1.13 ha;

d) The average mortality of *A. flavipes* adults directly increased in relation to the instar of the host *D. saccharalis*; and,

e) The borer presented a support capacity to *A. flavipes* parasitism from the 3rd instar on, being superior in the 5th and 6th instars.

INTRODUCTION

According to Moutia and Courtois[11], *Apanteles flavipes* was originally described by Cameron in 1891 in Poona, India. It was redescribed by Wilkinson in 1928. It is geographically distributed in India, Ceylon, Formosa, Japan, Australia, Malaysia and Mauritius.

This insect is a primary larval parasite of pyralids and noctuids. According to Gifford and Mann[7], it seems to be as proliferous on *Diatraea saccharalis* as on its original hosts. Its adaptation has been successful in Mauritius since 1952, in Reunion since 1964, in Antilles and Barbados since 1967 and in Saint Kitts since 1970 (Delattre[3]). The adaptation of this parasite has also been confirmed in Colombia (Gaviria[5,6]) and in Guyana (Des Vignes[4]).

In Brazil, the first positive results concerning the adaptation of *A. flavipes* were achieved in Alagoas. After 32 months of its introduction in the state (PLANALSUCAR[12]) the average parasitism by *A. flavipes* reached 8.38%, which represents 46.24% of the total parasitism in Alagoas. After a rapid adaptation in the state of Bahia, the average parasitism reached 10.97%, which is quite close to the one reached by the most effective parasite up to that time, *Metagonistylum minense* (Towns., 1927).

In spite of the success of the *A. flavipes* to control *D. saccharalis,* in the Northeast of Brazil its adaptation to the state of Sao Paulo conditions has been slower. This difficulty has been overcome by mass rearing of the insect by PLANALSUCAR laboratory or by the laboratories in Sugarmills, under the assistance of PLANALSUCAR technicians. In 1978, these laboratories produced and released approximately 50 million *A. flavipes.*

Due to the natural difficulties in establishing a biological control program in the Central-South region of Brazil, this program provided basic information concerning the parasite behavior following mass production and liberation, thus granting better chances of success.

This work was undertaken to determine, under field conditions in the region of Araras, state of Sao Paulo, the average dispersion of *A. flavipes* and the interaction model between this parasite and its host. Also evaluated was the rate of parasitism by *A. flavipes* supported by *D. saccharalis.*

MATERIAL AND METHOD

A. flavipes *dispersion*

This trial was carried out at Sao Joao Sugarmill, Araras, state of Sao Paulo.

The parasites were supplied by the Laboratory of Biological Control of the Southern Regional Co-ordination Service of PLANALSUCAR. Many of the 1,230 cocoons were kept in laboratory until adult emergence, after which they were transported and released in the fields.

From November 1978 to April 1979, 5 releases took place in previously selected blocks planted with the sugarcane variety NA 56-79 (plant cane). These blocks were far from one another, and *A. flavipes* did not occur naturally in these areas.

In each block, approximately 44,280 adults of *A. flavipes* were released. The sexual ratio of 1 male for 1.27 females resulted from the release of about 24,600 females.

A fortnight after releases, the biological material (hosts and live parasites) were collected, in circles, at the distances of 0-20, 20-40, 40-60, 60-80, 80-100, 100-120 and 120-140 meters from the release area. In each distance, in 8 places randomly chosen, 15 "biological forms" (larvae and pupae) were collected, totaling 120 biological forms per circle.

The collected material was brought to the laboratory, where it was separated and recorded. The live larvae were kept in plastic boxes, and fed with sugarcane bits, in order to make a selection of the healthy from the parasitized material possible. After 15 days, this material was reviewed, and eliminated after the data were recorded.

Finally, with the data from field surveys (*A. flavipes* cocoons recovered), the dispersion was calculated. This value was derived from a determined mean, where the number of recovered cocoons and the distances within which they were found were considered. The final value was achieved through the average of treatment means.

Area of discovery of the parasite A. flavipes *and expected population*

In order to obtain the data for this experiment, samples were taken from the same localities of the three last replications of the previous experiment.

A fortnight after the release of the parasites, 8 random samples were taken at the following distances: 0-20, 20-40, and 40-60 meters, from the area of release. In each of these circles, 120 stalks were taken (15 stalks per sample spot). From these all biological materials such as larvae and pupae, were removed. Near each sample area, the number of stalks per linear meter were counted (8 replications per circle) for an estimation of the average number of stalks per hectare.

Knowing the approximate number of stalks per hectare and the number of biological forms found in the 120 stalks examined per circle, it was possible to estimate the number (or density) of hosts subject to the attack of the parasite (N) and the number (or density) of non-parasitized hosts (S). To calculate the parasite area of discovery (A), the following Nicholson's mathematic model was used (Varley*et al*[13]):

$$a = \frac{1}{p} \log_e \frac{N}{S}$$

where p is the number of released parasites (female).

Knowing the parasite area of discovery, the following equation was applied:

$$P_n + 1 = N_n - \text{antilog}\left(\log N_n - \frac{ap_n}{2.3}\right)$$

where p_n and p_{n+1} are the successive parasite populations and N_n, the host population. The equation allows the estimation of the amount of searching parasites in the following generation.

Parasitism support by D. saccharalis *larvae*

Together with field trials, a trial under laboratory conditions (temperature of 27°C ±2) was also carried out to check the parasitism support of *D. saccharalis* to the attack of *A. flavipes.*

This experiment was made with 20 larvae from the 3rd to the 6th instar (totaling 80 larvae), reared on the slightly modified Hensley and Hammond's[9] artificial diet. Each lot of 80 larvae was considered a plot, and the experiment was replicated 10 times.

The larvae stung by the parasite were kept in plastic boxed with diet for feeding. This material was examined every day and the cocoons of *A. flavipes* collecteed. These cocoons were kept in plastic boxes for later observation on the percentage of efficiency $\left(\frac{\text{amount of cocoons} \times 100}{\text{total of stung larvae}}\right)$, number of days required for emergence of adults, and mean longevity of adults in days. The number of cocoons in each mass and the number of adults which have emerged was also determined.

In order to check the difference among the various instar, the variance analysis was used and the test of Tukey applied.

RESULTS AND DISCUSSIONS

A. flavipes *dispersion*

The data used for the dispersion calculation and the values achieved are shown in Table 1.

According to these results, it can be observed that the average of dispersion varied from 25.33 to 48.12 meters, resulting in an overall average of 34.38 meters.

Although this dispersion was small, embracing an area of approximately 0.4 ha, it can be considered an advantage in case of *A. flavipes.* Since the parasite has been introduced in the region and this small dispersion promoted the occurrence of mating, the presence of females in the subsequent generation was ensured because this insect presented a facultative haploid arrhenotokous parthe-

nogenesis. Therefore, these dispersion areas in the field could constitute initial spots

TABLE 1. Cocoons of *Apanteles flavipes* (Cameron) recovered in the field, per distance, replication, total and weighted mean (Araras, SP).

Distance in meters	Replications					TOTAL
	1	2	3	4	5	
0 – 20	9	18	15	16	11	69
20 – 40	4	4	8	6	4	26
40 – 60	1	4	5	3	0	13
60 – 80	0	0	1	1	0	2
80 – 100	0	0	0	2	0	2
100 – 120	0	0	1	2	0	3
120 – 140	0	0	1	2	0	3
Weighted Means	28.57	29.23	40.64	48.12	25.33	34.38

of distribution of the parasite, ensuring its adaptation in the region. On the other hand, if this parasite showed a high dispersion, it is most probable that after some generations the population would disappear from this area due to the lack of opportunity for mating. This information agreed with the work of DeBach and Bartlett[2] who considered this as an important factor for the introduction of a parasite in any area.

Based on the dispersion achieved the release of this insect shall be recommended to be made in locations approximately 70 meters apart in borer infested sugarcane areas.

Area of discovery of the parasite A. flavipes *and expected population.*

In order to measure the area of discovery of the parasite *A. flavipes,* the biological forms were sampled in the field, and the data were shown in Table 2.

To apply the Nicholson's formula, $a = \frac{I}{p} \log_e \frac{N}{S}$, the values of P, N and S were determined.

The determination of the value of P was based on data of Table 3, where it was determined that the average of adults resulting from cocoons was approximately 36 (35.94) individuals. Considering the sexual ratio 1:1.27, the result showed 20 females per cocoon. A total of 1,230 cocoons had been released, thus about 24,600 *A. flavipes* females were released (P).

To determine the values of N and S, the number of stalks per meter, was initially calculated. An estimated 145,379 stalks were in an area of approximately 1.13 ha. with a 60 meter radius.

One hundred eighty seven biological forms were present in 1,080 stalks examined (Table 2) while 25,172 hosts in 145,379 stalks were exposed to the attack of the parasite (N). If the same procedure was applied to figures in Table 2, it was possible to determine the number of non-parasited hosts (S), which amounted to 23,288 individuals.

TABLE 2. "Biological forms" examined in 120 stalks per distance, replication and total (Araras, SP).

Biological Material	Distances in meters and Replications									General
	0 – 20			20 – 40			40 – 60			
	1ª	2ª	3ª	1ª	2ª	3ª	1ª	2ª	3ª	Total
Healthy borers	14	11	21	15	18	21	19	16	15	150
live pup. *M. minense*	2	0	2	0	4	3	1	2	3	17
live pup. *P. claripalpis*	0	1	2	0	0	1	0	0	0	4
live cocoons *A. flavipes*	3	3	1	2	0	0	4	1	0	14
live pup. *Ipobracon* sp.	1	0	0	0	0	0	0	0	0	1
Borers with *Beauveria* sp.	0	0	1	1	0	0	0	0	1	3

Applying the Nicholson's formula, the value achieved was 0.031 m^2 (a), corresponding to the area of discovery of the parasite.

The next step was the calculation of the number of searching parasites in the following generation, which resulted in 1,838 cocoons ($P_n + 1$).

These results were achieved according to Nicholson's mathematic model, which supposes that there is no limitation in the oviposition, that the search is randomized and that the area of discovery is constant. However, since it is a new parasite still being introduced in the region, the amount of information concerning its behavior under field conditions is small, and perhaps a more detailed study on some aspects that may interfere in the parasite population such as host density, handling time (interval between the first host-parasite encounter and search being resumed – Holling[10]), density of the parasite, constant of interference (Hassel and Varley[8]) and possibly others, would better characterize the interaction model of parasite and host.

However, by the number of cocoons recovered in the field (Table 2), the

TABLE 3. Average number of cocoons, adults, average survival in days and % of efficiency of *Apanteles flavipes* (Cameron) achieved through inoculations of parasite on *Diatraea saccharalis* (Fabr.) larvae from the 3rd to the 6th instars. Statistical analysis (Araras, SP).

Instars	Replications Variants	1	2	3	4	5	6	7	8	9	10	MEAN	F	TUKEY (+) 5%
3º		30.60	31.71	46.10	43.86	36.10	42.38	41.33	44.00	37.14	50.16	40.34		
4º	Cocoons	34.00	33.75	36.27	40.80	26.13	46.25	34.83	45.29	47.87	39.43	38.46	ns	
5º		45.43	44.27	37.54	35.13	49.50	42.50	51.36	42.29	51.19	33.07	43.23		—
6º		36.19	34.45	33.87	35.47	39.57	45.00	45.29	40.58	53.07	39.67	40.32		
3º		27.80	28.43	44.20	38.86	32.00	38.50	38.00	41.00	33.14	47.66	36.96		
4º	Adults	28.75	29.13	27.82	34.90	21.00	41.81	32.17	39.57	45.20	37.00	33.74	ns	—
5º		38.14	40.07	27.00	29.56	46.58	36.30	43.36	26.86	45.88	25.67	35.94		
6º		28.94	29.36	25.07	24.73	33.14	37.82	36.64	28.58	50.14	30.87	32.53		
3º		2.10	2.95	2.78	2.56	2.03	2.39	2.89	2.04	3.31	2.11	2.52		b
4º	Survival	2.14	1.99	2.46	2.23	1.96	2.16	2.19	2.27	2.15	2.47	2.20	6.51**	ab
5º		1.97	1.73	2.25	2.17	1.77	2.28	2.34	1.89	1.94	2.31	2.06		a
6º		1.90	1.84	2.22	2.02	1.62	2.02	1.85	2.03	2.38	2.03	1.99		a
3º		25.00	35.00	50.00	35.00	50.00	40.00	45.00	10.00	35.00	30.00	35.00		a
4º	% Efficiency	40.00	40.00	55.00	50.00	40.00	80.00	30.00	35.00	75.00	35.00	48.00	19.94**	a
5º		70.00	75.00	65.00	80.00	60.00	50.00	70.00	70.00	80.00	75.00	69.50		b
6º		80.00	55.00	75.00	75.00	70.00	85.00	70.00	60.00	70.00	75.00	71.50		b

(+) The means followed by different letters are statistically different.

value of 1,885 cocoons of *A. flavipes* in the studied area (1.13 ha) can be calculated and this value was almost the same as the one achieved by the formula of Nicholson's model in 1938. It suggested that the parasite should present a behavior similar to the Chalcid parasite *Nasonia vitripennis* (Wirk), which according to DeBach and Smith[1], followed the same model.

Parasitism support by **D. saccharalis** *larvae*

Laboratory studies were conducted to determine the support capacity of *D. saccharalis* to the attack of *A. flavipes*, (data is shown in Table 3).

There were no significant differences between host instars studied in the production of parasite cocoons and adults, indicating a similar behaviour in any of these instars. However, when the longevity and percentage of parasitism were determined, some differences became evident. The mean longevity was shorter in individuals coming from the 5th and 6th instars, possibly due to the quality of food available for the parasite in different instars of the host. However this point deserves further investigation. The percent efficiency of the parasite was higher in host larvae of the 5th and 6th instars, indicating a better capacity of support of the host to the parasitism. This led us, inspite of the shorter longevity, to work in the laboratory with hosts of the 5th and 6th instars for the mass production of the parasite *A. flavipes.*

These results showed that in the field, *D. saccharalis* was susceptible to the parasitism by *A. flavipes* from the 3rd instar on.

CONCLUSIONS

The results of this work allowed the following conclusions:

a) The average dispersion of *A. flavipes* was 34.39 meters;

b) The area of discovery of the parasite *A. flavipes,* according to Nicholson's model was 0.031 m^2;

c) The population of searching parasites in the following generation, resulting from the release of approximately 24,600 *A. flavipes* females was 1,838 cocoons in an area of 1.13 ha;

d) The average longevity of *A. flavipes* adults decreased in relation to the instars of the host *D. saccharalis;*

e) The borer presented a support capacity to *A. flavipes* parasitism from the 3rd instar on, being superior in the 5th and 6th instars.

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ASPECTOS DE LA DINAMICA DE POBLACIONES DE *APANTELES FLAVIPES* (CAMERON) Y LA CAPACIDAD DE SUBSISTENCIA DE SU HOSPEDERO, *DIATRAEA SACCHARALIS* (FABR.)

P. S. M. Botelho, N. Macedo y A. C. Mendes

RESUMEN

El objetivo de este trabajo es el de calcular en condiciones de campo, la capacidad de dispersion y el patron de interaccion o comportamiento entre el parasito, *Apanteles flavipes* (Cameron, 1891) y el hospedero, *Diatraea saccharalis* (Fabr., 1794). Tambien conocer el poder de subsistencia de este hospedero en condiciones de laboratorio.

Los resultados obtenidos nos permiten llegar a las siguientes conclusiones:

a) La capacidad de dispersion promedio de *A. flavipes* fue de 34.38 metros.

b) El area de hallazgo del parasito *A. flavipes*, de acuerdo con el patron de Nicholson, fue de 0.031 m^2.

c) La poblacion de parasitos activos en la siguiente generacion, despues de haber liberado aproximadamente 24,600 hembras de *A. flavipes*, fue de 1838 capullos en una superficie de 1.13 has.

d) La mortalidad promedio de adultos de *A. flavipes* aumento con relacion al estadio de hospedero, *D. saccharalis*.

e) El barrenador tuvo un poder de subsistencia al parasitismo desde el tercer estadio, siendo superior en el quinto y sexto estadio.

TABLE 1. Life cycle pattern in relation to seasonal climate (35-year means)

	Long-term mean values											
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Mean max. temp. °C	26.0	28.1	30.7	32.9	32.8	32.8	32.4	32.7	31.0	30.1	28.2	25.6
Mean min. temp. °C	7.4	9.3	13.1	17.2	19.3	20.0	20.3	20.0	18.5	15.5	10.8	8.1
Mean Tem. °C	16.7	18.7	21.9	25.0	26.0	26.4	26.3	25.8	24.8	22.8	19.5	16.8
Rainfall mm	5	4	8	19	77	111	129	106	82	25	7	13
Mean RH%	59	55	52	52	59	64	69	72	70	67	64	63
Eggs							**	**	*	*		
Larvae	*	*	*	*	*	**	**	**	**	**	**	**
Prepupae	*	**	**									
Pupae		*	**									
Adults			*	*	**	**	**	**	*	*		

** Significant at 5% level*
** Significant at 1% level*

1.0 mm to 1.7 mm in width. During incubation they increase in size and become more spherical in shape, measuring 1.3 mm to 2.0 mm in diameter and becoming ivory white just prior to hatching. Sweeney[8] reported that females laid between 30 and 80 eggs each, and Symes[9] recorded 60 to 70 eggs per female. The incubation period varies from 8 to 21 days, depending on temperature and soil moisture. Eggs cannot survive exposure to the sun and are very susceptible to soil moisture stress, but they appear to be immune to the effects of excessive moisture.

(b) *The larvae* are typical white grubs in shape and appearance, being dirty white in colour with a pale brown head and assuming a bluish-grey colour when

actively feeding. There are three larval instars described by Sweeney[8] (Plate 1). Young first instar larvae first appear in late November, but the main hatching occur inclusively from December to March. The larvae are active for 5 to 8 months, during which time they are capable of erratic movement in the soil, mainly in search of food or to burrow deeper as the soil- dries out between irrigations. An earthen cell is formed by the larvae towards the end of the third instar, which is normally in July/August, and although they may remain quiescent in these cells for several weeks without change, they normally enter the pre-pupal stage within a week or two (Sweeney[8]).

(c) *The pupal* stage occurs from August to October following a period of 1 to 3 weeks as pre-pupae, but pupae tend to be most abundant in September and none have been found after mid-October. Cells are generally found in the vicinity of the stool at approximately the same depths as the feeding larvae, i.e. mainly within 30 cm of the soil surface.

(d) *The adult* beetles are shiny black in colour, measuring 14 to 20 mm in length and 6 to 10 mm wide, but they are genetally brown to dark brown soon after shedding their pupal skins. Most of them remain dormant in their cells until stimulated to emerge by the first rains of the season. Adults have been found feeding on cane on September, mainly in poorly drained areas, but it is not clear whether these are newly emerged adults or whether they represent a small residual population which has successfully over-wintered. The main build-up of adult populations appears to be dependent on the occurrence of rainfall, irrespective of normal irrigation schedules, which indicates that pupal cells in the fields are not usually formed in the irrigation furrows.

DAMAGE TO SUGARCANE

Adult damage

In young cane, the adults feed on new shoots just below the ground level, destroying the growing point and causing the central heart or the whole shoot to wither and die (Plates II and III). Such damage is readily visible in young ratoon cane 2 to 3 weeks after harvest, and the beetles are subsequently capable of completely destroying the primary stand within 2 to 3 months. Counts of "dead-hearts" made in a field of NCo 376 in December, 1978, showed that 78 8000/ shoots/ha had been destroyed within 3 weeks of harvest, and that shoot mortality had increased to 250 000/ha after 12 weeks. Compensatory tillering is insufficient to replace such loss, with the result that stands are drastically reduced, gaps develop as whole stools are destroyed, and yields are soon reduced to a level necessitating plowing and replanting (Plate IV).

Visible symptoms of damage diminish as the shoots get older, because the growing points are then above the ground and the beetles rarely bore right through the stems causing the tillers to die back. For this reason it was at first believed that

PLATE 1

PLATE 2

PLATE 3

PLATE 4

PLATE 5

PLATE 6

PLATE 7

damage was confined to young tillers in late-season crops, i.e. those harvested from September until the end of the cutting season early in December. Detailed surveys in 1978/79 revealed that beetles were actively feeding on cane of all ages, boring into both young and mature stalks and leaving a hole 1.0 to 1.5 cm in diameter at the base of the stalk leading into an enlarged chamber (Plates V VI, VII). Cane damaged in this way does not die, but the injury causes a serious growth check and loss of uniformity, which is generally the only indication of beetle activity until the stools are inspected below ground level.

There is considerable evidence to show that adults prefer to feed on young cane and that they only move into older cane when less young tillers are available i.e. towards the end of the cutting season. However, the pattern of infestation and damage lacks consistency, a fact which was also noted by Sweeney[8] in Swaziland, and it is common to find a heavily infested field adjacent to one almost entirely free from infestation. The adults tend to favour heavier soils, but although in 1978/79 they were more widespread on heavy black montmorillonite clays they were certainly not confined to them, causing damage on lighter textured soils but avoiding gravely and stony situations.

Larvae damage

While the main food of the first instar larvae is organic matter in the soil, the second instar, as it grows, feeds progressively more on green plant material, while the third instar feeds voraciously on the host plant (Sweeney[8]). The main food item is roots of all sizes, but later stage larvae will tunnel into the stool and crown, severing roots but also feeding on more solid material including the dead crown of the previous crop.

Damage is most obvious from February to August, and it can be devastating when populations are high. Extreme injury causes wilting and yellowing of leaves symptoms which are identical to those caused by soil moisture stress. Under such circumstances whole stools can easily be pulled out of the ground with little effort, because the root system has been mostly destroyed. However, there can be a high level of infestation without any obvious superficial effect to the cane, as individual stalks or stools seldom die.

DISTRIBUTION IN THE SOIL

Regular fortnightly samples were taken from a heavily infected field during the 1978/79 summer to study the vertical and lateral distribution of adults, larvae, and eggs. Typical counts are shown in Table 2, each value being the mean of 12 samples expressed as a percentage.

Data shown in Table 2 clearly indicate that the distribution of adults and larvae is closely associated with their feeding habits. The adults are present mainly in the vicinity of the stool, with relatively few being found in the interrow. In the

TABLE 2. Percentage distribution of adults, larvae and eggs

Month	Depth (cm)	Adults		Larvae		Eggs	
		In-row	Inter row	In-row	Inter row	In-row	Inter-row
Jan	0-15	100	0	57	13	88	0
	15-30	0	0	20	4	12	0
	30-45	0	0	6	0	0	0
Feb	0-15	90	10	37	14	55	0
	15-30	0	0	30	5	9	9
	30-45	0	0	10	4	18	9
Mar	0-15	60	20	64	9	100	0
	15-30	20	0	17	5	0	0
	30-45	0	0	3	2	0	0
Apr	0-15	0	0	58	10	0	0
	15-30	0	0	15	3	0	0
	30-45	0	0	11	3	0	0

case of larvae, 70 to 80% were found in the top 30 mm around the stool, with the rest scattered throughout the profile feeding on roots which are more widely distributed than the preferred food of the adults. Eggs were found mainly in the surface horizon of the cane row where adults were most numerous.

The distribution of adults and larvae in the soil varies markedly in relation to soil moisture conditions, with larvae tending to burrow deeper as the soil dries out between irrigations, and adults tending to migrate to wetter areas when the soil gets too dry near the surface.

EFFECT ON YIELD

It has not been possible to quantify the effects of *H. lucas* damage on yields of sugarcane in Rhodesia, simply because the problem has not been studied for a sufficient length of time. However, records from certain fields which were treated in 1976 after severe adult damage to young ratoons showed that yields dropped by 30 to 40% as a result of the damage before being restored to normal levels two years after treatment. Many other commercial fields, whose yield potential declined to 60-70% in recent years have been found to be heavily infested, although *H. lucas* may not have been the sole cause of the problems.

It has also been apparent that smut *(Ustilago scitaminea* Syd) levels have been considerably higher than normal in heavily infected fields, largely due to the increased susceptibility of late-formed tillers, and the general unthriftiness of the crop which makes it more prone to disease attack.

Severe losses in Swaziland have been attributed by Sweeney[8] to be due mainly to larval damage, with the most serious effects occurring in 3rd to 5th ratoons. He measured a mean loss of 55 tons/ha from 9 fields in the third ratoon, and recorded losses of almost 100 tons/ha in more seriously infected fields.

The evidence in Rhodesia indicates that such losses are possible, but only in areas which have been heavily infected for at least 2 to 3 years, and certainly not over the industry as a whole.

CONTROL MEASURES

Biological and cultural control

Diseases and predators of *Heteronychus* spp. were studied by Sweeney[8] who concluded that it was unlikely that any form of biological control would give permanent success because endemic insect parasites and predators were too scarce in cane fields.

Similarly, cultural practices cannot be expected to control the pest, although they could effectively reduce damage in susceptible areas. For example, damage tends to be more severe in poorly drained areas, where adults emerge early in the season to start feeding, and improved drainage in such areas would reduce insect damage in addition to providing other yield benefits.

Drying out after harvest by reducing the frequency of irrigation late in the season would have the effect of reducing adult activity, but the degree of desiccation required to kill adults and larvae would cause considerable stress to the cane. Eggs are more susceptible to desiccation, but the beetles will not lay eggs in dry soil, and rainfall during the main egg-laying period would reduce the chance of prolonged periods of drying-out. Other cultural practices, such as avoidance of alternate hosts, rotations, deep cultivations, light traps, etc., would all be beneficial, but none would be effective in significantly reducing the overall population.

Chemical control

The only immediate prospect of effective control of *Heteronychus lícas* is by the use of insecticides.

White grubs of various insect species have long been troublesome throughout the sugarcane-growing world and have been classified as major pests in many countries, with the result that a considerable amount of research has been conducted on methods of controlling these pests with insecticides.

In S. Queensland, the larvae of *Rhyparida* spp. beetles cause dead-heart symptoms similar to those caused by the adult *H. lícas* and they are effectively controlled with aldrin of BHC (Moller and Mungomery[6]). White grubs of several Melolonthid species also attack sugarcane in Queensland, and are generally control-

led with BHC, as dieldrin and aldrin have been shown to be ineffective (Wilson[11]). In India, BHC has also been shown effective against grubs of *Holotrichia* spp. (Avasthy[1]), while Bates[2] reported that in the Americas white grubs were no longer considered as major pests since the advent of chlorinated hydrocarbons, particularly BHC. Sweeney[8] recommended dieldrin for the control of *H. licas* in Swaziland, in preference to any other insecticide, but he did not conduct trials to compare the relative effectiveness of different compounds.

H. licas completes its life cycle underground and both adults and larvae are subterranean feeders. It is thus an extremely difficult pest to control because of the problem of getting a suitable insecticide into the soil where the insect is active. Soil treatment at the time of planting is the only practical means of applying insecticide to the main feeding zone, i.e. underground in the vicinity of the stool. Trials have been initiated in Rhodesia to compare various concentrations of a range of insecticides for pre-plant treatment, but it will be several years before these trials provide useful results. In the meantime, dieldrin is being used throughout the industry, applied either as a 50% W. P. or 15% E. C. at the rate of 2 kg/ha a.i. sprayed in a band over the planting furrow before the setts are covered back.

It has been shown by many workers that dieldrin incorporated in the soil has a residual effect of up to 4 years, so that treatment at planting should provide control for the plant crop and 2 to 3 ratoons. However, the Rhodesian crop cycle is 6 to 8 years and often longer, so that effective reapplication of insecticide to the established crop becomes necessary.

Considerable progress has been made by local farm equipment suppliers in developing suitable implements for applying insecticide below the surface for *H. licas* control, but in many cases such methods cannot be used and surface application is necessary.

Work in Swaziland has shown that when dieldrin is sprayed on the soil surface it has sufficient residual activity to prevent serious larval damage for at least 1 to 2 years. Surface spraying will not prevent adult damage, however, even though it will eventually kill the beetles, because the adults are capable of inflicting considerable damage to the cane between the time of contact with the dieldrin on the soil surface and eventual death. Sweeney[8] found that adults contacting the soil surface after surface spraying 1 to 3 months previously, died within a few days; if sprayed 5 to 9 months previously the beetles took 10 or more days to die; and when surface spraying was done 1½ to 2 years previously, mortality was erratic with only a 50% kill in 10 to 14 days.

Trials were initiated in Rhodesia in 1978 to study the effectiveness of surface applications of dieldrin and aldrin for control of *H. licas,* and results revealed valuable short-term effects.

Trial I. The second ratoon of a commercial field was harvested in November,

TABLE 3. Effect of treatments on adult and larvae populations and on shoot mortality (percentages)

Treatment	Adults	Larvae	Dead shoots
Control	100.0	100.0	100.0
Dieldrin @ 1.0 kg/ha a.i.	77.4	43.1	75.5
" 2.5 kg/ha a.i.	57.4	49.7	62.1
" 4.0 kg/ha a.i.	52.0	27.2	60.4
Aldrin @ 1.0 kg/ha a.i.	57.4	38.0	67.4
" 2.5 kg/ha a.i.	34.6	28.1	54.3
Ñ " 4.0 kg/ha a.i.	42.6	38.9	52.1
Banded over cane row	40.6	23.4	55.8
Banded over inter-row	70.6	54.0	71.4
Full cover spray	49.4	35.0	58.5

1978, and within 2 weeks extensive shoot mortality was evident as a result of adult beetle activity. A trial was conducted on the site, with treatments applied 3 weeks after harvest and comprising 3 levels each of dieldrin and aldrin (1,0; 2,5; and 4,0 kg/ha a.i.) applied either as (a) a band over the cane row, (b) a band over the inter-row, or (c) a full cover spray. Immediately after application the inter-row was cultivated and reshaped for furrow irrigation. Weekly sampling for insect counts, and weekly records of dead shoots were taken to provide a measure of insect activity. Results after 3 months are shown in Table 3, where cumulative totals have been expressed as percentages of control values.

The most noteworthy short-term effects were that (a) aldrin provided better control than dieldrin, (b) 1 kg/ha was less effective than 2.5 or 4.0 kg/ha. (c) inter-row application was less effective than the other methods, irrespective of insecticide treatment, (d) banding over the cane row was no less effective than a full cover spray at twice the cost, and (e) control of larvae was better than control of adults.

Trial II. Four levels of dieldrin were compared in a second trial, with the insecticide applied as a banded surface spray over the cane row in an area where damage was so severe that four months after the trial was started it had to be abandoned because of the loss of stand caused by *H. licas* damage. Table 4 shows cumulative counts for the first three months after treatments were applied, expressed as percentages of control values.

The results from both these trials clearly show that surface applications of dieldrin and aldrin, even at low concentrations, reduce egg-laying and larval populations more effectively than they reduce adult population,s even though the larvae

TABLE 4. Effect of treatments on insect populations and shoot mortality (percentages)

Treatment	Adults	Larvae	Eggs	Dead shoots
Control	100	100	100	100
Dieldrin @				
1.0 kg/ha a.i.	80	47	97	75
2.0 kg/ha a.i.	64	44	76	63
3.0 kg/ha a.i.	46	32	44	69
4.0 kg/ha a.i.	48	39	35	59

do not come into contact with the insecticide. Sweeney[8] found out that egg-laying by fertile adult females was inhibited even by sub-lethal doses of dieldrin, and that any progeny produced survived for only a very short period. It must also be recognized that adult counts did not accurately reflect their response to treatments, because adults are constantly moving and many that have contacted insecticides will die elsewhere and be replaced by others.

DISCUSSION

H. licas is an endemic insect in Rhodesia, as it is in Swaziland where environmental conditions are very similar, and under natural conditions it feeds in wet areas on grasses, sedges, reeds, and bulrushes, provided these plants are not waterlogged. However, the normal semi-arid conditions prevailing in the natural bush restrict the excessive multiplication of the insect because suitable breeding sites dry out during the winter months and seriously interfere with the life cycle during the larval phase.

The available evidence clearly shows that the build-up of *H. licas* from 1974 to 1978 was closely associated with a series of seasons with abnormally high rainfall, following a period of several years with rainfall below average. This is shown in the following table in which seasonal totals and deviations from the long-term (35-year) mean of 586 mm are presented.

Season	Rain (mm)	Dev. from mean (mm)	Season	Rain (mm)	Dev. from mean (mm)
1966/67	252	−334	1972/73	266	−320
1967/68	261	−325	1973/74	875	+289
1968/69	479	−107	1974/75	734	+148
1969/70	394	−192	1975/76	848	+262
1970/71	330	−256	1976/77	721	+135
1971/72	728	+142	1977/78	1048	+462

The increase in *H. licas* populations is attributed, therefore, to (a) the series of wet seasons which created a greater number of suitable habitats and breeding sites in the natural bush, (b) many of these breeding sites remaining wet throughout the winter without the adverse effect of a drying cycle, and (c) the development of ever-increasing populations in cane fields which offer the insect optimum conditions all the year round. The result of these factors was an increase in the reproductive rate far in excess of normal, causing a minor pest to assume major pest status in a relatively short period of time.

The Rhodesian sugarcane industry was not affected by any serious insect pest before the advent of *H. licas*. During the years when rapid expansion of cane production took place (1957-1965), it was customary on virgin soils to apply dieldrin or aldrin in the planting furrow for termite control, but this practice was discontinued with the progress of time. In recent years there have been no insecticides applied to the cane fields, and the sudden increase in *H. licas* has been unchecked by residual chemicals.

It has thus been necessary to initiate control measures from scratch, with economic considerations largely dictating the extent to which they are adopted. Growers have been strongly advised to treat all newly planted fields, and in the case of established fields to give priority to areas of poor drainage, to heavier soil types, and to young ratoons. Chemical methods cannot be expected to eradicate an endemic pest, and the research projects which have now been initiated are aimed at reducing populations in order to minimize damage. In time, results of these trials will serve to reinforce or modify the basic control measures already advised, all of which are soundly based on experience gained elsewhere on the control of insects with similar feeding habits.

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INFORME SOBRE EL DAÑO QUE OCASIONA EL ESCARABAJO* DINASTIDO, *HETERONYCHUS LICAS* (KLUG.) EN CAÑA DE AZUCAR

K. E. Cackett

RESUMEN

Heteronychus licas siempre había sido considerada una plaga menor sin importancia economica para la industria azucarera de Rhodesia, hasta que un inesperado aumento de población del insecto resultó en un daño significativo que coincidió con un aumento de la precipitacion entre los años de 1974 y 1978. Los adultos se alimentan bajo tierra de vástagos y tallos de todas las edades durante los meses humedos del verano, mientras que las larvas lo hacen de raicillas y yemas subterráneas a fines del verano y durante el invierno. Las pérdidas estan en acorde con las "fallas" en siembras y mortalidad de cepas habiendo la necesidad de "voltear" el campo y resembrarlo cuando el daño es muy severo.

El trabajo trata tambien sobre el ciclo de vida del insecto, y se ofrecen resultados preliminares de investigaciones que se han iniciado sobre el estudio biologico y el control de la plaga. Se aconseja el uso de Dieldrin por el momento hasta que se tengan los resultados de pruebas comparativas con otros insecticidas y sus efectos residuales en condiciones locales.

*Mayate, Chicharon, Gallego, etc.

LOSSES FROM *MAHANARVA POSTICATA* IN SUGARCANE

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ABSTRACT

The losses of cane as well as sugar yields due to infestation by the leaf froghopper *Mahanarva posticata* (Stal, 1855), a very important pest in Pernambuco and Alagoas states, were determined through two controlled field tests.

The experiments included two treatments: a plot exposed to natural infestation and another freed from infestation by dusting of insecticide. Six rows, each ten meters long of cane variety CB 52-41 were used in each plot for 1977 and five rows, each ten meters long of cane variety Co 853 were used in each plot in 1978, with 10 replications in both experiments.

In the first test, in which all cane, treated and untreated, was exposed to attck prior to treatment, a respective decrease for the cane and sugar yields of 11.2 and 14.9% were observed. The population per stalk was 6.43 of the third and fourth instars, 3.75 of the fifth instar and 0.81 for the adults. In the second test, the respective decreases obtained for the cane and sugar yields were 13.9 and 16.9%. The average leaf froghopper population was 2.87 nymphs and 0.68 adults per stalk.

INTRODUCTION

The Brazilian sugar production has been affected by numerous pest, the most prominent being the moth borers *Diatraea saccharalis* (F.), *Diatraea flavipennella* (Box), the giant moth borer *Castnia licus* (Drury), the leaf froghopper *Mahanarva posticata* (Stal) and the root froghopper *Mahanarva fimbriolata* (Stal).

Actually, in Northeast Brazil, and particularly in the states of Pernambuco and Alagoas, the leaf froghopper *Mahanarva posticata* (Stal) (Hom: Cercopidae) (Fig. 1 and 2) is considered to be the pest of greatest economic value. The sugar plantations of these states cover approximately 700,000 hectares and were respon-

sible for a production of 2,400,000 metric tons of sugar in the harvest of 1978/79 (I.A.A.[13]).

M. posticata belongs to the Cercopidae family, of which there are other

FIGURE 1. Nymphs and spittle of the leaf froghopper *Mahanarva posticata (Stal) on sugar* cane stalks.

representatives also of economic importance to other sugar growing regions of the world. *M posticata* provokes the red streak blight on the sugarcane leaves (Fig. 3).

In 1889 the froghopper *Aeneolamia varia saccharina* (Distant) was first discovered by J. H. Hart who described the outbreak of blight in the Chaguanas district in Trinidad and believed it to be due to the said insect (Williams[15]).

The formation of leaf blight is mainly caused by the feeding of the adults (Williams[15]. Withycombe[16] Guagliumi[7,9], Hagley[11], Fewkes[6] Marques[14]).

The total destruction of leaf tissue resulting from a single puncture during feeding of an adult *A. varia saccharina* is reported to sometimes exceed half a square inch (Williams[15]). This injury indicated the possibility that a viral infection, a toxin or an enzyme is introduced into the leaf during feeding (Fewkes and Cutler[3]).

Present in the salivary gland secretion of the adults and fifth instar nymphs of *A. varia saccharina,* are amino acids and enzymes. The artificial inoculations of solutions of the amino acids and enzymes produced a greater or lesser amount of red streaking typical of early blight development. A very weak lipase appeared to be present in the nymphal salivary glands, and it is probable that a sufficient quantity of the enzyme did not accumulate at the site of toxic action for its effects to be observed (Hagley[12]).

The size and speed of development of a blight mark probably depends upon the duration and extent of the insect's feeding, as well as on the physiological state of the same plant. The loss of photosynthetic tissue causes a severe retardation in the growth of the plant. Thin, stunted internodes are characteristic of blight-damaged sugar cane. In addition, the juice quality may be adversely affected (Fewkes[6]).

Fewkes and Buxo[4] conducting tests with controls to protect plots of cane against the froghopper *A. varia saccharina,* found an increase of 9.88 to 32.38 tons of cane per hectare in the treated plots, and in relation to the sugar yield, they mentioned an increase of up to 3.63 tons of sugar per hectare. Albert[1], referring to the *M. posticata,* mentioned that the losses are sensitive and are capable of reducing the production from 30 to 50% and diminishing the content of saccharose in the juice.

FIGURE 2. The leaf froghopper *Mahanarva posticata* (Stal). Male and female. (Extracted from Guagliumi[8]).

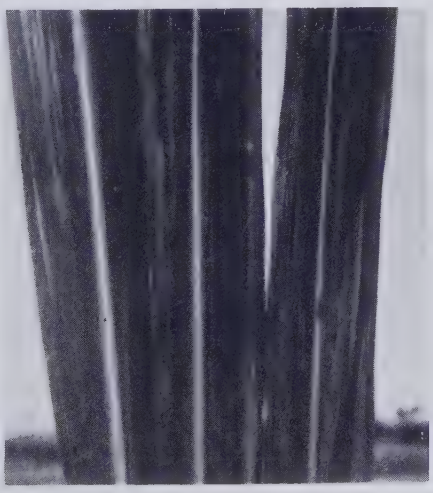

FIGURE 3. Blight streaks caused by feeding adults of *Mahanarva posticata* (Stal).

CODECAP in Guagliumi[10], confirmed a respective loss of 18.5 and 17.0% for the cane and sugar from the CB 45.3 variety and 16.5% in sugar for the Co331 variety, due to the attack of *M. posticata.*

The degrees of pest infestation responsible for the alluded losses have not yet been determined. For this reason, this work aims to find out the cane and sugar losses caused by *M. posticata,* using the informations from field works done on sugar plantations in Pernambuco, Brazil.

MATERIALS AND METHODS

Two experiments were carried out in the years of 1977 and 1978 in the fields of the Barreiros Central Sugar Mill in Pernambuco, Brazil. These consisted of two treatments with ten replications, in a randomized blocks design. Each plot was 10 meters long with 6 rows in 1977 and 5 rows in 1978.

The treatments consisted of: a) plots free from any system of control where counts of the leaf froghopper nymphs and adults were made in ten stalks in each plots every 15 days. During the period of sampling, the nymphs of the first instar were not considered. b) plots controlled alternately with insecticides applied every 15 days, according to the manufacturers's recommendations, using BHC 3%, Dicarban 85 and Perfektan EC 20. In the experiments of 1977 the plots were 30 meters apart and the CB 52-41 lowland sugarcane variety was used. Control was begun when the plants were 5 months, 15 days old and presented an average infestation of 1.48 adults, 4.5 nymphs of the fifth instar and 19.8 nymphs of the third and fourth instar per stalk. Consequently, the plots had been exposed to the first brood of the pest. In 1978, the plots were 50 meters apart and the Co 853 lowland sugarcane variety was used. Control was started when the plants were a month old.

The average population of *M. posticata* occurring on the plots was calculated for a period of six months in 1977; and for six months and 15 days in 1978.

Finally, the plots were harvested and weighed, and samples were taken to analyze the sugar and calculate the tons of cane and pol yield per hectare.

RESULTS

In 1977, the average population of *M. posticata* was registered and is shown in Table 1. The analysis of the variation of tons of cane and pol yield per hectare is shown in Table 2.

In tons of cane per acre the difference between treatments was not significant at the 5% level. However, taking the averages of the treated plots and non-treated plots, there is an increase of 11.2% in the cane production in the areas without froghoppers.

mined either in kilograms and/or tons per hectare. This factor multiplied by the average intensity, and by the harvested area, results in the total loss of recoverable sugar. The loss factor of recoverable sugar determined for each 1% intensity in cultivar H32-8560 is 4.551 kg/ha or 0.004551 t/ha.

The percentages of fiber and reducing sugars are greater in the cane portion damaged by the borer-phytopathogen complex whereas the percentages of pol, purity and recoverable sugars are less in comparison with the unbored cane portion.

The sugar loss in percent and in kg/ton cane, is directly proportional to the percentage of bored internodes, with a highly significant coefficient of correlation ($r = 0.99$).

A linear regression between the percentage of length damaged and the percentage of intensity, with a highly significant coefficient of correlation ($r = 0.96$) has been determined.

It has been found out that as the number of harvests increase the percentage of bored internodes decrease, thus, the recoverable sugar loss in kg/t cane also decreases.

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ESTIMACION DE LAS PERDIDAS DE AZUCAR RECUPERABLE CAUSADA POR EL COMPLEJO "BARRENO" *(DIATRAEA SACCHARALIS* FABR.), Y PATOGENOS EN EL CULTIVAR H32-8560

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RESUMEN

En la Cooperativa Agraria de Produccion Casa Grande Ltda. No. 32, durante las safras 1972-1977, se determino las perdidas de azucar recuperable causada por el complejo "barreno" *Diatraea saccharalis* (Fabr.) y patogenos mediante muestreos intensivos de campos con cañas maduras, mas a la cosecha. Para ello se analizo separadamente la calidad de las muestras tanto de las porciones de caña dañadas como de las sana.

El promedio estimado de la perdida de azucar recuperable, en cinco zafras fue de 0.025 kg/t de caña. para 1% de entrenudos barrenados.

Se determino una regresion lineal de la perdida de azucar recuperable (en kg/t caña y en porciento) en funcion del porcentaje de entrenudos barrenados (Intensidad). con un coeficiente de correlacion altamente significativo (r = 0.99). Del mismo modo se encontró una regresion lineal del porcentaje de longitud dañada en funcion del porcentaje de entrenudos barrenados, con un coeficente de correlacion altamente significativo (r = 0.96).

Tambien se encontró que a medida que aumenta el numero de cortes el porcentaje de entrenudos barrenados decrece.

Para evaluaciones de caracter practico, se ha determinado un factor de perdida para cada 1% de Intensidad. Este factor multiplicado por el porcentaje promedio de Intensidad y por la superficie cosechada, da como resultado, la pérdida total de azúcar recuperable, expresada en toneladas por hectareas, siendo este factor de 0.004551 t/ha.

THE ASSESSMENT OF ECONOMIC DAMAGE CAUSED BY *AENEOLAMIA VARIA SACCHARINA* DIST. (FROGHOPPER) ON SUGAR CANE IN TRINIDAD

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ABSTRACT

During 1976–1979 attempts were made to assess the economic importance of the froghopper, *Aeneolamia varia saccharina* (Dist.) on sugar cane in Trinidad. Five factorial experiments designed for this purpose did not provide meaningful results. However, observations at Waterloo, Estate 27A on strips of sugar cane showed that the blight produced by adult froghopper attack resulted in reduction of sugar cane brix, pol, juice purity, cane weight and fiber. There was difficulty in measuring blight and the author suggested that the froghopper day criterion may probably be more suitable for damage/loss assessment. New economic considerations for insecticide application have been suggested since Fairrie formula used in the past for assessment of the cost of a ton of sugar proved to be inadequate for the prevailing situation.

INTRODUCTION

Merry *et al.*[3] and Fewkes and Buxo[1] showed that the froghopper (*Aeneolamina varia saccharina* Dist.) infested sugarcane and caused reduction in yields. However, at present, there is still no information on yield losses as related to froghopper population.

The froghopper is at present controlled by the aerial application of insecticides. This study was the first of a series of studies intended to determine the damage levels or cost/benefit threshold necessary for the improvement of froghopper control strategy.

MATERIALS AND METHODS

During the period 1976-1979 observations on froghopper population and damage levels were conducted at sugar cane fields in Waterloo (fields 27A, 352 and 344), Felicite (9 W 9 and 7 W 8), Exchange (331 and 319) and Edinburgh (R3 and 4) using a 2 x 2 x 2 factorial experiment with a randomized complete block design. All fields except Waterloo (352 and 344) were planted with variety HJ 5741. Waterloo 352 and 344 were planted with B41227.

At Waterloo (27A) there were six replications for each treatment and each plot was 1/40 of an acre in size. In addition, a five acre portion of the same field was later divided into four strips with visually distinct blighted levels namely unblighted, slightly blighted, moderately blighted and badly blighted.

The other four sites, Waterloo (352 and 344), Felicite, Exchange and Edinburgh, were unreplicated using a 2 x 2 x 2 factorial experiments set out in a randomized complete block design Plot size was 1/4 of an acre.

The eight treatment plots of the factorial experiment were denoted as follows: 2, 3, 4, 2 + 3, 2 + 4, 3 + 4, 2 + 3 + 4 and 1.

2 = 2nd brood spraying with Unden once per week
3 = 3rd brood spraying with Unden once per week
4 = 4th brood spraying with Unden once per week
2 + 3 = 2nd + 3rd brood spraying with Unden once per week
2 + 4 = 2nd + 4th brood spraying with Unden once per week
3 + 4 = 3rd + 4th brood spraying with Unden once per week
2 + 3 + 4 = 2nd + 3rd + 4th brood spraying with Unden once per week
1 = Control in which no sprays were applied

A weekly estimate of the numbers of froghoppers per stool and stem was made in all plots using a stratified random sampling technique, (Morris[5]). The froghoppers on 20 canes were counted at the east, west, north, south and center of each treatment. A composite sample was then made by counting the froghoppers on four canes from each of these points.

Towards the end of the fourth brood a weekly estimate of the blighted area of the third or fourth leaf from top visible dewlap (TVD) was measured on graph paper and recorded from random samples of cane leaves collected from the strips of sugar cane at Waterloo 27A.

At harvest, the total number of canes, the number of millable stalks, the weight of cane per plot, the height and width of canes at the base and top, and the number of bored joints were recorded from 0.0063 acre portions of the sugar cane fields.

Juice analysis was performed on plot samples using 5 samples of 10 canes from Estate 27A and 10 samples of 10 canes from all other experiments using the method described by Tanimoto[6].

RESULTS

The results on strips of cane in field 27A have been presented in Tables 1, 2 and 3 and which show that froghopper attack caused a reduction of sugarcane brix, pol, juice purity, cane weight and fiber. In 1976-1977 there was a high degree

TABLE 1. Relationship between insect population, blight development, cane quality and cane weight on 2nd ratoon cane variety HJ 5741, at Waterloo Estate 27

	Unblighted	Treatment blighted	Moderately blighted	Badly blighted
Total No. froghopper days in broods 2, 3 + 4	21,800	22,680	21,420	36,540
No of froghopper days in brood 2	6,720	9,240	10,080	23,100
No. of froghopper days in brood 3	2,520	3,780	4,200	3,360
No. of froghopper days in brood 4	12,600	9,660	7,140	10,080
% Blighted leaf area	24	25	24	26
Weight of cane/acre (lb)	17,280	21,920	16,960	10,720
Brix	17.24	17.40	17.40	17.40
Juice purity	92.05	91.51	92.45	91.58
Pol % cane	15.12	15.20	13.68	13.83
Fiber + % cane	73.19	72.00	67.86	68.11
TC/TS 10 months cane	6.66	6.75	7.11	7.24
TC/TS 12 months cane	6.62	6.60	7.33	7.24
TC/A	7.71	9.79	8.91	4.79
TS/A	1.16	1.48	1.22	0.66
! % *Diatraea* bored joints	5.2	5.4	5.1	5.0

of association between the number of froghopper days and the TC/Ts of ten months cane ($r = 0.92$), and 12 months cane ($r = 0.82$). There was also a high degree of association between number of froghopper days and TC/A ($r = 0.89$) and TS/A ($r = -0.89$).

In general, the interplot differences in number and weight of canes per unit area were so great that the differences caused by froghopper attack were easily masked except in the strips of cane at Estate 27A. In addition, the blight development was only pronounced at Felicite and Waterloo 27A. However, at Felicite the blight development was very uniform throughout all treatments. The only outstanding differences found was with the strips of cane at Waterloo 27A, which developed distinct blighted levels (referred to as unblighted, slightly blighted,

TABLE 2. Relationship between percent blighted leaf and sugarcane quality at Waterloo Estate (27A) on 3rd ratoon cane of variety HJ 5741.

	Treatment			
	Unblighted	Slightly blighted	Moderately blighted	Badly blighted
% Blighted leaf area 4th leaf from TVD	4	43	61	87
Brix	17.2	15.1	15.4	15.1
Juice purity	84.3	85.1	84.2	83.5
Pol % cane	12.5	11.1	11.3	11.2
Fiber % cane	13.5	13.4	12.8	11.5
TC/TS 12 months cane	8.0	9.2	8.9	8.9

TABLE 3. Relationship between insect population, blighted development, cane quality and cane weight, at Waterloo Estate (27A) on 4th ratoon of sugar cane variety HJ5741.

	Treatment	
	Control	Badly Blighted
Total froghopper days in broods 2, 3, & 4	21,975	62,625
No. of froghopper days in brood 1	1,125	—
No. of froghopper days in brood 2	6,975	18,750
No. of froghopper days in brood 3	10,200	10,125
No. of froghopper days in brood 4	4,300	33,750
% Blighted leaf area 4th leaf from TVD	65.2	100
Brix	16.6	15.6
Juice purity	83.9	82.1
Pol % cane	12.3	11.2
Fiber % cane	12.6	12.6
TC/TS	8.4	9.0
TC/A	13.4	9.8
TS/A	1.6	1.09
Average no. of canes/stool at harvest	11.8	9.28
Average no. millable canes/stool at harvest	2.0	1.46

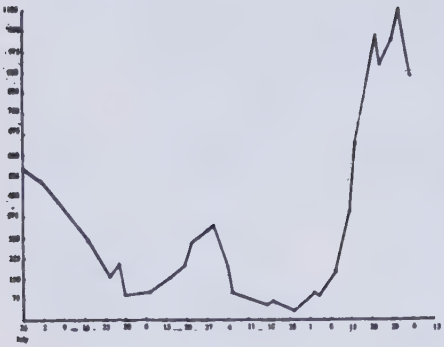

FIGURE 1a. Number of froghopper days at Waterloo 27A. Badly blighted plot of 4th ratoon sugar cane during 1978 - 1979.

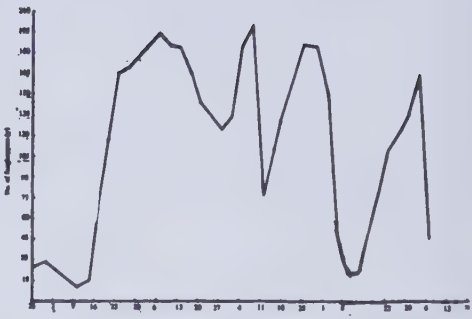

FIGURE 1b. Number of froghopper days at Waterloo 27A control plot of 4th ratoon sugar cane during 1978 - 1979.

moderately blighted and badly blighted).

Visual observations showed that the reduction in length of the upper-most joints of sugar cane stalks was responsible for the reduced height of the moderately blighted and badly blighted sugarcane stalks in 1976-1977. There was also a reduction in thickness of the uppermost joints but no measurements were recorded.

DISCUSSIONS AND CONCLUSIONS

The observed deterioration of sugarcane can be largely explained by the toxaemia or blight produced by froghopper adult attack which resulted in a reduction of photosynthetic activity (Hagley[4]). Attack by the nymphs on the roots of sugarcane also caused reduction in yield but no attempt has been made to assess this loss since the present froghopper control program is primarily geared to suppress adult froghopper populations (Gopee[2]).

It should be noted that the attempt to assess the level of blight was time consuming and difficult and the froghopper day criterion will probably be a better indicator of losses sustained. Again, experiments designed to assess the importance of nutrient levels and physical quality of the soil in blight prevention or retardation have not been completed.

Fewkes and Buxo[1] used the cost of a ton of sugar (Fairrie formula) in making an assessment of economic benefits to be derived from froghopper control. In Trinidad, the cost of producing a ton of sugar is greater than the price of a ton of sugar on the international market and further economic considerations are to be made before recommendations on insecticide application thresholds can be derived.

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LA EVALUACION DEL DAÑO ECONOMICO DE *AENEOLAMIA VARIA SACCHARINA* DIST. (MOSCA PINTA) EN CAÑA DE AZUCAR EN TRINIDAD

W. G. des Vignes

RESUMEN

Durante los años de 1976 a 1979 se ha tratado de evaluar la importancia economica de los daños que la mosca pinta* causa a la caña de azucar en Trinidad. Cinco experimentos factoriales orientados hacia el esclarecimiento de lo anterior no ofrecieron los resultados deseados. Sin embargo, observaciones realizadas en Waterloo Campo 27A, demostraron que la quemazon o tizon producido por el adulto de la mosca pinta, produce una reduccion en el brix, pol, pureza, peso y contenido de fibra en caña. Existen ciertas dificultades en medir esta quemazon, y el autor sugiere que el criterio normal utilizado localmente es el mas apropiado para evaluar el daño/perdida. Se sugieren nuevas consideraciones para la aplicacion de insecticidas, ya que la formula Fairrie para calcular el costo de una tonelada de azúcar, no es la adecuada en la actualidad.

*Candelilla, Salivazo, Salvita, Cigarrinha.

BASIC STUDIES ON RESISTANCE OF SUGARCANE VARIETIES TO THE SUGARCANE BORER IN BRAZIL

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ABSTRACT

Two different trials were carried out to determine the resistance of sugarcane varieties to the sugarcane borer *Diatraea saccharalis* (F. 1974) in Brazil.

The first trial, installed in two locations included 37 clones in the FT_4 phase of selection from the PLANALSUCAR sugarcane breeding program and 8 commercial varieties, with known reaction to the borer attack as checks. NCo 310 and US 16-94 varieties were also included. Borer infestation data, expressed as Intensity of Infestation (I.I.), were obtained from both locations for plant cane (in 1977) and ratoon (in 1978).

In the second trial, 35 progenies were used. These were obtained through polycrosses from 8 parent varieties. In 1977 borer Intensity of Infestation (I.I.) was determined at harvest time on samples of .20 stalks.

Measurement of clonal repeatibility and association between parent and progeny behavior allowed the following conclusions:

1. Screening clones for resistance to borer infestation in the FT_4 phase from PLANALSUCAR sugarcane breeding program (polycross system) could be practiced with a good chance of progress.

2. Genetic variance prevailed over other interaction components of variance, and it was apparent that clonal response to borer infestation, between locations and between plant cane and 1st ratoon was

relatively consistent, thus allowing selection in only one experimental area, and only on plant cane, when a large number of replications are used.

3. The high correlation coefficient obtained (r = 0.86) between parent and progeny behavior indicated that there is an additive genetic control for the character of resistance.

INTRODUCTION

The sugarcane borer, *Diatraea saccharalis* (F., 1794) is the most injurious insect attacking sugarcane in the southern region of Brazil, where more than one million hectares of sugarcane are grown. It was estimated that for the 1978/79 harvest, this insect caused a crop loss of US$25 million. The damage was caused by tunneling inside the stalks, causing dead top plants, breakage of stalks and loss in cane weight and sugar yields.

The PLANALSUCAR National Program of Sugarcane Improvement, through the southern regional Section of Entomology is conducting a program for biological control of the borer using introduced parasite, *Apanteles flavipes*. By special techniques, it has been possible to multiply wasps in the laboratory and release in borer infested sugarcane areas more than 100 million wasps per year. In spite of the success of this program, other studies must be developed in an endeavour to reduce losses caused by the sugarcane borer.

The use of resistant varieties of sugarcane, besides being recognized as a desirable method of controlling the borer, has none of the more obvious disadvantages of other methods of pest control, such as extra cost to the grower, possible harmful insecticide residues and damage to beneficial insects. Therefore, it is also an ideal method to be developed together with classic biological control.

Resistance of sugarcane to the sugarcane borer as stated by Mathes and Charpentier[3] may result from one or more of the following factors: (1) unattractiveness of host-plant to moths for egg deposition, (2) plant characters unfavourable to the establishment of borers in the plant, (3) plant characters which inhibit or retard borer development, and (4) tolerance, or the ability to yield well in spite of a high infestation.

The same authors found it difficult to determined any single character confering resistance to borers from numerous interlocking characters. There are two main schools of thought on methods of breeding borer-resistant varieties of sugarcane: one calls for identifying the individual traits that are responsible for the resistance, and breeding these into a good agronomic variety; and the second advocates accepting as parent varieties, the few that show marked resistance, without concern for what traits make these varieties resistant.

Mathes and Charpentier's[3] opinion is that the first method is only applicable

where not more than two characters are involved, and many resistant characters are agronomically undesirable.

Since occasionally some good agronomic varieties are resistant, but do not have characters specifically associated with resistance, there is now a trend to use cane parents with marked resistance without regard to the cause of resistance (Azzi[1]).

Based upon these opinions and pursuing the same general objectives of the borer resistance investigations carried out by U.S.D.A. and the Louisiana Agricultural Experiment Station, which are: (1) to discover cane varieties resistant to the borer to be used as parental breeding material, (2) to determine the resistance of promising new varieties which may later be released to growers, and (3) to determine the resistance or susceptibility of the more common commercial varieties; that observations and studies on resistance of sugarcane to sugar cane borer in our region were established.

Viator and Henderson[4] conducted a study to determine the genetic behavior of resistance to the sugarcane borer. They found out that environment had a moderately strong effect on the expression of resistance to borer infestation in the progeny; there was a large number of continuously varying classes; and measures of clonal repeatibility for resistance to infestation in the progeny were moderately high, indicating that selection in a breeding program for this trait could be initiated as early as the "first line trials".

Macedo[2] worked for 3 consecutive years with 16 commercial varieties in trials carried out at four different sugarmills. He found a degree of resistance to *D. saccharalis* in some studied varieties that surpassed the influence of year, place and period of survey. Progeny behavior of some varieties was also studied, leading to the conclusion that characters inducing higher or lower attack of that pest were inherited.

The following are the results of two different trials that were carried out to determine (1) the possibilities of progress in resistance to borer infestation screening clones in the FT_4 phase of selection from PLANALSUCAR sugarcane breeding program, (2) influence of interactions among varieties, location of planting, and cutting period, (3) coefficient of heritability in progenies from a polycross system, where the behavior of one parent to the pest is known.

MATERIALS AND METHODS

First Trial

The materials used included 37 clones in the FT_4 phase of selection from the PLANALSUCAR sugarcane breeding program, here designated as A_1, A_2, A_3, A_4, A_5, A_6, B_1, B_2, B_3, C_1, C_2, D_1, D_2, E_1, E_2, E_3, F G, H, I, J, K, L, M, N, O, P

Q, R, S, T, U, V, W, X, Y, and Z, and 8 commercial varieties, five with a moderate degree of resistance (IAC 48/65, CB 45-155, CB 56-171, IAC 50/134 and Co 775); 2 with a high degree of susceptibility (IAC 52/150 and CP 51-22); and one intermediate (Co 740), all previously classified according to its reaction to borer attack and serving as checks in this experiment.

Also included were NCo 310, the most resistant commercial variety in the USA and US 16-94, recommended for parents (Mathes and Charpentier[3])

A ten-meter row plot was established for each of the clones and varieties at five random replications, totaling 235 plots per location. Two locations quite far from one another, were chosen: SAO MARTHINHO MILL (L_1) (21° 09' southern latitude, 48° 01 western longitude and 500 altitude) and BANDEIRANTES MILL (L_2) (23% 10' - southern latitude, 47° 23' - western longitude and 400 altitude).

To determine the Intensity of Infestation (I. I.) 20 talks from each plot, were sampled at harvest time totaling 100 stalks per variety or clone, on which the total joints, and borer-damage joints were counted.

Borer infestation data were obtained from both locations, for plant cane (in 1977) and ratoon (in 1978). Data were expressed as the percentage of joints showing external evidence of infestation, referred to henceforth as Intensity of Infestation.

The results of I. I. for the 37 clones, with 2 cuttings, 2 locations and 5 replications were submitted to variance analysis for:

i) individual analysis per cutting in each location;
ii) joint analysis of cuttings in each location;
iii) joint analysis over cuttings and location.

Coefficients of heritability in the broad sense were estimated on a clone mean basis, as follows:

i) $$h^2_{m_1} = \frac{\sigma^2 g}{\sigma^2 g + \frac{\sigma^2 e}{5}}$$

ii) $$h^2_{m_2} = \frac{\sigma^2 g}{\sigma^2 g + \frac{\sigma^2 a}{5} + \frac{\sigma^2 b}{10}}$$

iii) $$h^2_{m_3} = \frac{\sigma^2 g}{\frac{\sigma^2 a}{10} + \frac{\sigma^2 b}{20}}$$

where:

σ^2_g = genetic variance among clones;

σ^2_e = experimental error variance for type (i) analysis;

σ^2_a, σ^2_b = experimental error variances relative to plots and sub-plots in split-plot analysis of types (ii) and (iii):

Average I. I. for the checks and selected clones were set together to provide a reasonably accurate assessment of the previous analysis.

Second Trial

A polycross method of breeding was used to obtain progenies from several parent varieties, among which were CB 40-13, CB 49-260, Co 740, Co 775, CP 51-22, IAC 50/134, IAC 51/250 and NA 56-62.

Seedpieces from 35 progenies from these parent varieties were planted in plots 2 rows x three-meters without replication. The 35 progenies were established, in parental rows without randomization. This experiment was carried out at PLANALSUCAR Southern Experimental Station, Araras - SP., in 1977.

Intensity of Infestation (I. I.) were determined at harvest time on samples of 20 stalks from each plot. Data were submitted to variance analysis and linear correlation between previous I. I. of parent varieties and the I. I. average of their progenies was calculated.

RESULTS AND DISCUSSIONS

First Trial

Data on infestation (I. I.) of the experimental clones and check varieties from experimental area and cutting period are presented in Table 1.

An individual analysis per cutting in each location is shown in Table 2.

Although the coefficients of variation were relatively high, the coefficients of heritability ($h^2_{m_1}$: 71%, 69%, 69%, 49%) were quite high, suggesting a probable progress in FT_4 clonal selection for resistance to borer.

The high $h^2 m_1$ values obtained were partly due to on adequate number of replications.

The reasonable correlation coefficients obtained (r_{12} = 0.564 and 0.533) between the clonal behavior from one cutting to another in both experimental areas, suggest the possibility of selection only in plant cane. On the other hand, this information is readily apparent from values shown in Table 2, where the high resistance in some clones (C, K, L, and C_2), as well as the high susceptibility in other R, A_6, A_2, A_5, U and W) are evidence in both cuttings and locations.

TABLE 1. Intensity of Infestation of clones and check varieties for each experimental area and cutting period. (1977-1978)

Variety	Sso Martinho Mill			Bandoirantes Mill			General Total
	1st Cut	2nd Cut	Means	1st Cut	2nd Cut	Means	
C_1	6.87	5.21	6.04	6.12	10.26	8.19	7.11
E	5.79	9.81	7.80	7.12	10.52	8.82	8.31
L	7.42	9.85	8.63	6.82	10.28	8.55	8.59
C_2	6.19	9.40	7.79	9.83	9.79	9.31	8.80
O	7.67	10.61	9.14	10.41	10.31	10.36	9.75
A_4	6.10	14.30	10.20	5.59	14.37	9.98	10.09
J	8.7	9.92	8.99	11.41	11.39	11.40	10.20
Z	8.85	8.17	8.51	13.00	11.40	12.20	10.35
E_1	7.90	15.48	11.69	8.00	11.41	9.70	10.70
I	7.84	10.68	9.26	11.70	13.15	12.42	10.84
X	8.78	8.23	8.50	10.15	16.73	13.44	10.97
F	8.39	12.34	10.36	9.96	13.73	11.84	11.10
G	7.17	16.74	11.95	9.15	11.58	10 36	11.16
Y	10.44	7.57	9.00	11.02	17.23	14.12	11.56
Q	8.53	10.12	9.32	11.20	16.75	13.97	11.65
E_1	8.83	10.82	9.82	12.15	15.19	13.67	11.74
A_1	9.67	13.77	11.72	9.74	14.14	11.94	11.83
B_3	10.27	13.58	11.92	12.10	10.57	11.83	11.89
E_2	9.61	13.58	11.59	11.52	13.17	12.34	11.97
B_2	10.40	11.98	11.19	11.05	15.04	13.04	12.11
E_3	10.28	14.66	12.47	10.61	13.08	11.84	12.15
V	8.84	15.08	11.96	13.02	12.26	12.64	12.30
D_1	9.64	12.19	10.91	14.56	13.21	13.88	12.40
T	10.47	16.40	13.57	10.88	12.05	11.46	12.52
A_3	7.28	16.17	11.72	11.57	16.30	13.93	12.83
S	9.82	11.73	10.77	16.86	15.14	16.00	13.39
M	9.46	19.47	14.46	12.92	12.12	12.52	13.49
N	8.50	11.08	9.79	19.08	15.59	17.33	13.56
N	10.44	20.43	15.43	14.65	11.64	13.14	14.29
D_2	12.81	16.20	14.50	14.12	15.71	14.91	14.71
F	9.88	18.95	14.41	17.24	13.99	15.61	15.01
R	10.81	14.08	12.44	15.14	20.38	17.76	15.10
A_6	11.19	21.18	16.18	12.25	12.37	14.31	15.24
A_2	14.24	13.77	14.00	17.81	16.12	16.96	15.48
A_5	13.00	20.10	16.55	14.82	15.16	14.99	15.77
U	13.20	17.27	15.23	21.59	18.83	20.16	17.70
W	13.92	25.99	19.95	18.28	16.63	17.45	18.53
IAC 48/65	6.99	7.93	7.46	8.83	9.87	9.35	8.40
CB 45-155	5.46	7.85	6.65	9.92	11.09	10.50	8.58
CB 56-171	8.04	9.49	8.76	6.90	9.99	8.44	8.60
Co 775	8.81	9.47	9.14	7.75	9.20	8.47	8.80
Co 740	9.01	10.18	0.59	6.77	14.04	10.40	10.00
IAC 50/134	9.13	15.51	12.32	5.73	9.93	7.83	10.07
NCo 310	9.77	13.04	11.40	9.70	12.97	11.33	11.37
US 16-94	11.46	19.13	15.29	14.01	15.77	14.89	15.09
IAC 52/150	12.28	21.48	16.88	18.16	14.74	16.45	16.66
CP 51-22	15.06	24.21	19.63	12.48	15.89	14.18	16.91

TABLE 2. Mean squares for clones (MST) and error (MSE) and pertinent parameters. (1978)

	Sao Martinho Mill L_1		Bandeirantes Mill L_2	
	Cuttings		Cuttings	
	C_1	C_2	C_1	C_2
MST	22.57	96.20	66.47	34.66
MSE	6.51	30.22	20.33	18.11
m	9.40	13.70	12.30	13.80
C. V.	27%	40%	37%	31%
σ^2_g	3.20	13.20	9.20	3.30
h^2m_2	71%	69%	69%	48%
r_{12}	0.564		0.533	

L_1, L_2 = localities C_1, C_2 = cuttings

TABLE 3. Mean squares, their expectations and h^2m_2 estimates of the joint analysis of cuttings within localities, concerning borer infestation. (1978).

S.V.	D.F.	MS L_1	MS L_1	E/MS
T	36	85.7**	76.1**	$\sigma^2_b + 2\sigma^2_a + 10\sigma^2_g$
Ea	148	18.4	17.8	$\sigma^2_b + 2\sigma^2_a$
C	1	1687.5**	221.4**	$\sigma^2_b + 5\sigma^2_{gc} + 185V_C$
Cxt	36	33.1**	25.0	$\sigma^2_b + 5\sigma^2_{gc}$
Eb	148	18.3	20.7	σ^2_b
h^2m_2		78.5%	76.6%	

T = Clones
C = Cuttings
L_1 = Sao Martinho Hill
L_2 = Bandeirantes Hill

A joint analysis of cuttings in each location is shown in Table 3.

Statistical analysis of the data revealed that the clonal behavior was significantly different and, in spite of there being a significant interaction between cuttings x clones (CxT) at location I, it should not be a problem in selection however, due to the reasonable correlation between both cuttings.

The coefficient of heritability increased as expected because it refers to clonal selection among means of 10 data ($h^2_{m_2}$. However, the increase in the $h^2_{m_2}$ compared with those estimated for locations was not so high. This suggests that it is possible to work on only one cutting to select resistant clones with reliable results between cuttings. Direct examination of the data in Table 1 also shows that clones gave similar reactions between cuttings.

A joint analysis of cuttings and locations is shown in Table 4.

An examination of the estimated variances reveals that the error among plots, EB, is higher than other variance components. This reinforces the need of

TABLE 4. Mean squares, their expectations and significance for the joint analysis over cuttings and localities concerning borer infestation. (1978).

S.V.	D.F.	M.S.	E/MS
L	1	410.4**	$^2b + 2\sigma^2_a + 10\sigma^2_{tl} + 370V$
T	36	127.8**	$\sigma^2b + 2\sigma^2_a + 20\sigma^2_t$
LxT	36	34.1**	$\sigma^2b + 2\sigma^2_a + 10\sigma^2_{t1}$
Ea	296	18.1	$\sigma^2b + 2\sigma^2_a$
C	1	1565.7**	$\sigma^2b + 10\sigma^2_{ct} + 370V_c$
CxL	1	343.2**	$\sigma^2b + 5\sigma^2_{clt} + 185V_{cl}$
CxT	36	24.5ns	$\sigma^2b + 10\sigma^2_{ct}$
CxLxT	36	33.6*	$\sigma^2b + 5\sigma^2_{clt}$
Eb	296	19.5	σ^2b

L = *Localities*
C = *Cuttings*
T = *Clones*

using adequate numbers of replications in the kind of experiment. The contribution of the $o^2 b$ was reduced for clonal selection effects by the number of data (20) taken from each clone. In fact:

$$h^2_{m_3} = \frac{\sigma^2_g}{\sigma^2_g \quad \frac{\sigma^2_a}{10} + \frac{\sigma^2_b}{20}} = 0.858$$

This is an optimistic result from breeding purposes since, despite the significant clone x location interaction $h^2_{m_3} = 0.858$, it indicates the possibility of selecting clones favorable to both localities. On the other hand, it is not very much higher than the intra-location $h^2_{m_2}$ estimates – (0.785 and 0.766) for each location. Once more, these results suggest the possibility of selecting for resistance in only one experimental area, using only one cutting period.

Excluding the σ^2_a error variance, the genetic variance (σ^2_g) prevailed over other components of variance. Variance deriving from location x cutting interaction is quite less than the genetic variance which agrees with the considerations made before.

While the three-way intercation ($\sigma^2_{t_{1C}}$) was significant it will probably not be of consequence in clonal selection. In fact, the behavior of the clones in the two areas and cuttings was very similar.

Thus, despite the significant F value for interactions LxT, CxL, CxT and CxLxT, the genetic variance was strong enough and clonal repeatibility of reaction to borer infestation was enough that screening clones for resistance to borer infestation in the FT_4 phase of PLANALSUCAR sugarcane breeding program could be practiced with a good chance of progress, even working at only one experimental area and only on plant cane.

Also, it is apparent from the wide range among samples that the number of replications used was important.

Second Trial

Table 5 shows the results of I.I. for the 35 progenies of each parent CB 40-13, CB 49-260, Co 775, Co 740, CP 51-22, IAC 50/134, IAC 51/205 and NA 56-62.

Analysis of variance showed significant differences among the average behavior of progenies. However, the coefficient of variation was quite high (cv = 41.56), indicating a large number of countinuously varying classes within progenies. Table 6 shows the I.I. average for the parents from previous trials and I.I. average for the progenies obtained through polycross.

A correlation coefficient of 0.86 between parent and progeny behavior was obtained indicating that there is a genetic control for the character of resistance. Fig. 1 also shows the relationship between the I. I. in parents and the average I. I. of the progenies. Also the high correlation (2r = 0.86) between parent and progeny behavior indicates that there is an additive genetic control for the resistant traits.

TABLE 5. Intensity of Infestation for the 35 progenies of each parent variety. (1977)

Varieties / Progenies	CB 40-13 I.I.	CB 49-260 I.I.	Co 740 I.I.	Co 775 I.I.	CP 51-22 I.I.	IAC 50/134 I.I.	IAC 51/205 I.I.	NA 56-62 I.I.
F_1	9.09	7.04	5.55	3.01	11.68	8.73	7.56	12.95
F_2	6.96	3.40	8.11	5.08	17.71	11.93	7.05	11.31
F_3	6.80	7.39	6.74	1.93	17.64	1.82	12.94	1.92
F_4	7.03	5.32	13.57	7.69	14.56	8.81	15.29	13.01
F_5	9.82	9.82	4.66	5.88	13.81	4.85	12.77	4.94
F_6	7.15	9.44	2.87	7.47	13.77	4.08	8.12	6.79
F_7	7.87	9.16	9.75	10.93	12.53	4.83	6.41	9.28
F_8	8.00	9.20	9.63	5.32	16.45	5.84	10.60	7.16
F_9	4.86	3.53	9.34	2.86	22.55	10.30	11.81	4.79
F_{10}	6.89	8.89	8.92	5.46	20.16	4.83	10.00	6.83
F_{11}	4.49	4.95	6.22	5.85	8.87	3.90	8.35	6.42
F_{12}	5.44	10.65	10.54	6.11	7.50	4.50	14.81	4.68
F_{13}	3.99	6.25	12.40	8.16	6.95	12.16	11.57	2.74
F_{14}	4.89	11.36	13.35	6.23	6.57	12.38	14.09	5.81
F_{15}	6.53	7.96	11.42	5.63	14.82	11.96	11.41	4.80
F_{16}	9.58	3.98	12.32	7.65	6.60	9.35	4.69	3.35
F_{17}	8.76	4.78	15.94	15.02	5.78	4.72	10.73	6.29
F_{18}	6.63	7.63	15.82	14.46	4.78	8.16	3.16	12.01
F_{19}	10.49	4.19	11.95	9.37	12.62	4.76	6.64	24.78
F_{20}	10.13	10.81	11.77	5.12	6.14	10.93	6.92	6.40
F_{21}	7.76	9.84	12.10	5.22	12.02	5.01	8.68	6.80
F_{22}	8.70	7.73	10.62	6.92	12.97	6.99	8.31	6.30
F_{23}	11.54	11.45	10.12	7.35	12.31	9.94	14.89	6.25
F_{24}	6.95	6.38	8.43	8.8	16.41	10.31	8.99	5.46
F_{25}	9.28	8.96	5.31	5.72	11.61	3.39	17.53	14.41
F_{26}	14.06	6.99	8.87	5.93	23.00	7.28	2.72	7.87
F_{27}	11.73	5.12	3.21	12.05	13.21	3.62	5.54	6.98
F_{28}	9.89	8.27	6.72	5.86	16.09	11.65	3.57	11.02
F_{29}	15.36	6.50	3.67	10.54	14.06	2.05	6.99	4.22
F_{30}	18.86	6.53	12.04	12.19	10.55	5.48	19.49	3.01
F_{31}	11.45	9.00	10.26	7.90	11.66	7.58	7.86	8.47
F_{32}	12.36	6.21	T.36	8.63	12.38	10.28	8.89	9.62
F_{33}	11.19	11.19	10.37	10.57	12.50	16.34	10.00	13.10
F_{34}	12.40	6.41	14.97	0.92	9.09	9.94	6.49	14.25
F_{35}	10.15	7.28	7.49	2.63	13.82	10.30	17.39	0.97
MEANS	9.05	7.53	9.49	7.13	12.66	7.68	9.77	7.85

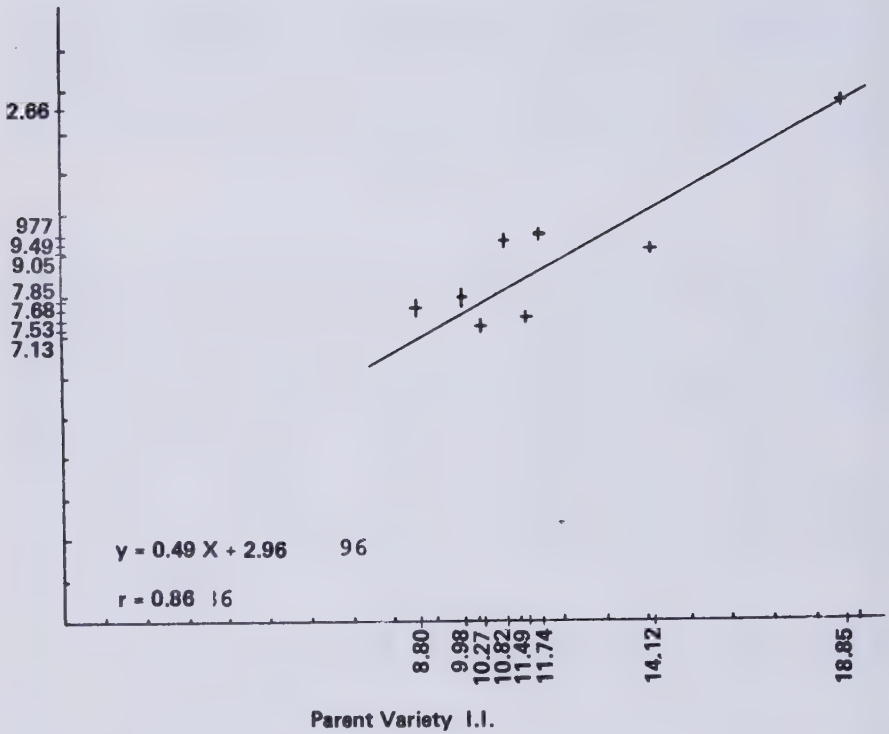

FIGURE 1. Relationship between parent varieties and progenies, for average I. I. of *D. saccharalis.*

TABLE 6. Response to borer infestation of parents and progenies, obtained through polycross. (1978).

	Parents* (I.I.)	Means of Progenies (I. I.)
CB 40-13	14.12	9.05
CB 49-260	11.49	7.53
Co 740	10.82	9.49
Co 775	10.27	7.13
CP 51-22	18.85	12.66
IAC 50/134	8.80	7.68
IAC 51/205	11.74	9.77
NA 56-62	9.98	7.85

(*) These data were obtained from previous trial.

CONCLUSIONS

1. Screening clones for resistance to the sugarcane borer, *Diatraea saccharalis* (F., 1794) in the FT_4 phase from PLANALSUCAR sugarcane breeding program (polycross system) could be practiced with a good chance of progress;

2. Genetic variance prevailed over other interaction components of variance, and it was apparent that clonal repeatibility of reaction to borer infestation between locations and between plant cane and 1st ratoon, was relatively high, allowing selection in only one experimental area, and only on plant cane, when a large number of replications were used;

3. The high correlation coefficient obtained ($r = 0.86$) between parent and progeny behavior indicated that there is an additive genetic control for the character resistance.

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ESTUDIOS BASICOS EN EL BRASIL SOBRE LA RESISTENCIA VARIETAL AL BARRENADOR DE LA CAÑA DE AZUCAR

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RESUMEN

Se realizaron dos pruebas diferentes en el Brasil con el objeto de

conocer ciertos conceptos basicos sobre la resistencia de variedades de caña de azucar al barrenador. Uno de los experimentos localizados en dos lugares bastante distantes uno del otro, constaba de 37 clones de la seleccion FT_4 del programa de cruzamientos de PLANALSUCAR, con un comportamiento conocido en cuanto a resistancia al barrenador. Fueron tambien incluidas las variedades N Co. 310 y U.S. 16-94. Los datos de infestacion expresados como Intensidad de Infestacion (I.I.), se obtuvieron de los dos lugares en caña de planta (1977) y retoños (1978).

En la segunda prueba se usaron 35 hibridos obtenidos mediante cruzas multiples, de cada uno de los 8 progenitores. La I.I. del barrenador fue determinada con muestras de 20 tallos tomados durante la zafra en 1977.

Considerando los datos obtenidos mediante replicas clonales y asociacion entre progenitor e hibrido, se llegaron a las siguientes conclusiones:

1. Se pueden seleccionar clones de acuerdo con su resistencia al barrenador, Diatraea saccharalis (F., 1974), en la fase FT_4 del programa de cruzamientos (sistema cruzas multiples) de PLANALSUCAR, con muy buenas posibilidades de exito.
2. La variante genetica prevaleció sobre todas las otras, y aparentemente los datos obtenidos entre los 2 lugares y entre las cañas de planta y retoños, respaldado por el gran numero de replicas nos indican que esta practica se puede llevar a cabo en un solo lote experimental y en caña de planta exclusivamente.
3. El alto coeficiente de correlacion obtenido ($r = 0.86$) en el comportamiento entre progenitor e hibrido, indica que se puede ejercer cierto control geneco en cuanto a resistencia de la variedad al barrenador.

POPULATION APPROACH TO SELECTION OF VARIETIES FOR SUGARCANE BEETLE BORER RESISTANCE

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ABSTRACT

Sixteen *Saccharum officinarum* **cultivars resistant to sugarcane beetle borer,** *Rhabdoscelus obscurus* **Boisd. were obtained using the principle of population approach to variety selection.**

The method could also be used to screen varieties for rat damage resistance. The resistant varieties thus obtained can also be utilized as source of gemplasm for breeding purposes.

The damage caused by the larval stage of the beetle borer increases fiber % fresh weight and decreases Brix of fresh juice.

INTRODUCTION

The sugarcane beetle borer, *Rhabdoscelus obscurus* Boised was first introduced into Fiji through Australia in the 1890s, (Veitch 1919[3]). During the early days as many as three million insects were collected in the Nausori Mill area alone (Knox[3]). The sugarcane beetle borer can be found in all parts of the sugarcane growing areas of Fiji.

The tunnelling and ingestion of the internal tissues by the grub stage of the beetle borer destroy much of the interior of the cane and in some cases the infestation is so heavy as to render the cane stalks worthless. This results in a marked reduction in the yield of sugar. The loss of sugar is, however, not only confined to the tissue actually consumed, but also by the susceptibility of such cane to break and lodge thus allowing further infestation and rotting. The secondary infestation of worms, ants, bacteria and fungi accelerates deterioration. It has also been observed that borer infestation is very closely ralated to rat damage. The rat eaten stalk affords easy oviposition for the adult female weevil.

After successfully using the population method by Daniels *et al*[1;2] for selecting varieties for commercial release, it was felt that these principles could be applied in selecting sugarcane varieties for beetle borer resistance. It was that the continuous propagation of clones showing resistance in the test population could

eliminate the susceptible clones.

MATERIALS AND METHODS

Two experiments were conducted at Labasa and Rarawai mill centers using the population approach for selections. These were located at known sugarcane beetle borer infested areas.

A foundation population of some 356 *S. officinarum* clones from LF70 series were used for these trials. Homer, Mana Ragnar, Mali and Vomo were planted as standard varieties. Ragnar and Homer were used as high sugar yielding varieties, Vomo and Mana as medium sugar varieties and Mali as low sugar variety. As for beetle borer damage assessment, Homer and Mana were used as susceptible standards, Ragnar as moderately resistant and Mali and Vomo as resistant.

The test varieties were planted in single stools replicated twice. The distance between stools was 45 cm in rows 1 meter apart. The varieties were allowed to stool freely. Cultivation was normal and adequate fertilizer was applied.

The beetle borer population was supplemented by collecting adult beetles with the aid of 'split bundle traps'. An average of twenty females and five males were 'dumped' per stool. Split bundle traps were prepared by splitting 45 cm of sugarcane stalk lengthwise and wrapping about 10 to 15 such split pieces with newspaper with the ends being open. These were placed in borer infested fields and collected after 4 to 6 days. This method yielded a large number of adult beetles of both sexes.

One hundred stalks of each standard variety were brixed and regression analysis done against the established ratings of these varieties. The discard level for brix was set at one rating lower than the lower rating obtained for any standard variety.The brix results of the two replications of the test varieties were averaged and the rating determined. The ratings used were 0 to 9 scale as described by Daniels *et al*[2] where 0 was used for high sugar varieties and 9 for low sugar varieties.

The discard level for beetle borer infestation was determined on a percentage basis. The same set of standard varieties were used for this purpose. Each standard was sorted out into two piles. "Pile' 'A' consisted of stalks which were apparently clean. Stalks from Pile 'B' were split lengthwise and examined for borer damage. The infested stalks were added on to Pile 'A'. Percent borer infestation was expressed by the number of infested and uninfested stalks against the 100 stalk samples taken.

The discard level was based at the average percentage obtained for Mali and Vomo, the two resistant varieties. As for the test varieties, the total number of stalk of the two replications were taken into account. Varieties which had acceptable brix rating were used for percent beetle borer determination. The selected

varieties were replanted for further evaluation. The experiments were continued for four years.

Random eighteen stalk sample of eleven varieties were also analysed for % fibre and brix at the small mill. The cane stalks were fibrated using a Jeffco cutter/grinder and juice obtained to determined 0 brix and fiber (Stevenson *et al*[4]).

RESULTS

Table 1 presents the selection of varieties over the four years that the trials have been in progress.

TABLE 1. Number of varieties selected

1975	1976	1977	1978
326	111	23	16

During the 1978 processing of the trial, the sixteen varieties planted were found to be almost free from any beetle borer damage. Only one stalk from one replication of variety LF70-1561 was found to have the last basal internode damage.

Table 2 shows the % fiber and brix analysis carried out on eleven varieties selected at random, constituting about 10% of the total population of 111 during 1976 harvest.

TABLE 2. Small Analysis – % Fiber

Variety	% Bored stalks	Bored	Clean	Increase in % fiber	Bored	Clean	% decrease in brix
Ragnar	50	15.2	12.4	2.7	15.7	20.5	4.8
LF70-458	80	11.0	9.5	1.5	12.0	20.0	8.0
LF70-466	100	10.8	9.7	1.1	9.0	20.0	11.0
LF70-1370	86	11.7	10.4	1.3	15.4	16.8	1.4
LF70-1405	77	19.2	14.5	4.7	17.4	21.0	3.6
LF70-1452	85	12.7	11.5	1.2	14.8	22.0	7.2
LF70-1454	75	13.3	13.2	0.1	15.6	19.4	3.8
LF70-1521	35	16.7	11.9	4.8	12.0	20.0	8.0
LF70-1594	60	14.5	14.5		9.0	18.0	9.0
LF70-1650	100	15.5	13.0	3.2	15.0	20.0	5.0
LF70-1754	50	12.5	12.3	.2	16.0	19.0	3.0

DISCUSSIONS

The population approach provides an opportunity for screening large numbers of clones for objective, metrical, measurable characteristics at all stages. In the current experiments the technique to develop beetle borer resistance in commercial sugarcane was used. As shown on Table 1, the susceptible clones were eliminated and the resistant ones left to demonstrate their superiority at subsequent stages. The population method also provides a cheap and ready selection method to eliminate susceptible or mediocre varieties.

The damage caused by beetle borer infestation generally increased the % fiber as compared to total fresh weight. This is mainly due to the fact that the grub or larval stage of the sugarcane beetle chews fresh tissue leaving dry and dead fibrous tissue after ingestion. This ingestion of internal tissue also renders the rind dry and hard.

The decrease in juice is also a direct result of ingestion of sugarcane tissue by the beetle borer. The infested portions of the sugarcane stalk dries out. In certain cases the stalk breaks at the point of infestation and dries out. This causes the brix to decline depending on the degree of infestation and tunnelling. Loss of sugar is due to the increase in fiber and secondary infestation. The secondary infestation also leads to increases in dextran and other polysaccharides, thus increasing the imputing load during the processing in the mill.

The population approach as outlined in this paper can also be used for assessing resistance to rat damage. The selected resistant clones can be profitably used as germplasm for breeding purposes.

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EL CONCEPTO DE LA POBLACION DEL ESCARABAJO BARRENADOR DE LA CAÑA DE AZUCAR COMO UN MEDIO PARA LA SELECCION DE VARIEDADES RESISTENTES.

S. C. Prasad, J. Subarmaniam y A. S. Masilaca

RESUMEN

El principio del concepto de poblacion para la seleccion de variedades puede tambien ser utilizado para la obtencion de cañas resistentes al escarabajo o mayate barrenador, *Rhabdoscelus obscurus* Boisd.

Los experimentos realizados han dado como resultados la obtencion de 16 formas resistentes del *Saccharum officinarum.* El daño causado por la larva del barrenador aumenta el porciento de fibra en el peso fresco de la caña, y disminuye los solidos solubles en el jugo fresco.

Este metodo se podria tambien utilizar en la seleccion de variedades resistentes a daños de roedores. Las cañas asi obtenidas podrian utilizarse como progenitores potenciales en programas de cruzamientos.

POTENTIAL BIO-CONTROL AGENT AGAINST SUGARCANE STEM BORERS FOUND IN NEGROS ISLAND, PHILIPPINES

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ABSTRACT

Four species of *Trichogrammatoidea* occuring in Negros Island, Philippines are described. Two species were of genus *Trichogramma*, and two species of *Trichogrammatoidea*. These four species of Trichogrammatidae are potential biological control agents *Tetramoera schistaceana* Sn. and *Chilotraea infuscatellus* Sn. *Trichogramma australicum* Girault, *Trichogramma chilotraea*, and *Trichogrammatoidea* sp. can be mass-reared in the laboratory on eggs of *Corcyra cephalonica* Stainton, while *Trichogrammatoidea nana* Zehnt. is specific on eggs of *Tetramoera schistaceana* in the field and laboratory. In the field, it was observed that the most efficient egg parasites against *Chilotraea infuscatellus* is *Trichogramma chilotraea* with 76.5 percent parasitism and *Trichogrammatoidea nana* against *Tetramoera schistaceana* with 91 percent parasitism.

INTRODUCTION

Sugarcane stem borers, *Tetramoera schistaceana* sn. and *Chilotraea infuscatellus* Sn. are the most destructive pests of sugarcane in the Philippines. The damage of these borers start from the early stage of growth to maturity of the plant. Approximately 8 percent of the annual crop was damaged by sugarcane stem borers (Espino[4]). In Negros, sugarcane fields with heavy borer damage were replanted (Estioko and Reyes[5]).

Chemicals were extensively used to control these pests. However, spraying with insecticides gave unsatisfactory results. The same was reported by Gallo *et al*[3]. This is attributed to the fact that borers bore into the stalk of the plant so that non-systemic insecticides could not kill them.

The failure of modern insecticides opened the way to biological control. Beneficial insects of family Trichogrammatidae were imported from Formosa, Japan, and U. S. A. to control lepidopterous pests of rice, corn, and sugarcane (Baltazar[2]).

So far, of the three imported Trichogrammatids: *Trichogramma australicum*

Girault, *T. japonicum* Ashmead, and *T. minutum* Riley, only *T. australicum* was found established in sugarcane fields against sugarcane stem borers (Alba[1]). Alba further reported that by natural parasitism, 15.69% of the total egg population of *Tetramoera schistaceana* were parasitized by *Trichogramma.*

Field recovery of *Trichogramma* indicates the potential of these egg parasites as biological control agent against sugarcane stem borers. Flanders and Quednau[6] believe that *Trichogramma* as biological entities are important in the control of lepidopterous pests. Likewise, Souza[10] reported the advantages in using natural enemies in the control of pests.

The purpose of this paper is to provide future basis for systematic work and biological control program against sugarcane stem borers.

EXPERIMENTAL PROCEDURES

Parasitized eggs of *Tetramoera schistaceana* and *C. infuscatellus* were collected from sugarcane fields in Negros Island for emergence. Four species of Trichogrammatidae were found parasitizing eggs of the above mentioned sugarcane stem borers. Two species were of the genus *Trichogramma* and two species were of genus *Trichogrammatoidea.* Among the four species of Trichogrammatidae identified, *T. australicum* was the imported egg parasite and the other three are believed to be native egg parasites of sugarcane stem borers.

Identification of specimens was based on the key to some arrhenotokous *Trichogramma* spp. (Nagarkatti and Nagaraja[7,8,9]).

Species of Trichogrammatidae collected were reared on eggs of *Corcyra cephalonica* Stainton, *Tetramoera schistaceana,* and *Chilotraea infuscatellus* in the laboratory for possible changes on its morphological and taxomic characteristics on different host.

RESULTS AND DISCUSSION

Description of Species of Trichogrammatidea

It is difficult to base identification of species of Trichogrammatidae on the external morphology of the insect. Nagarkatti and Nagaraja[7,8] used the male genitalia as the most reliable diagnostic characteristic. In this study it was observed that the four species of Trichogrammatidae possess pronounced differences in their genitalia.

List of some distinguishing characteristics that differentiate genera *Trichogramma* from *Trichogrammatoidea* are shown in Table 1.

1. *Trichogramma australicum.* Adult male is shorter than the female 0.48 mm long, 0.23 mm wide. Chrome yellow in colour with dark pigmented abdominal

segment. Antennae slender tapering hairs ranging from 32-36 with unsegmented flagellum (Plate 1 Fig. 1). Forewing 0.47 mm long, 0.25 mm wide with short oblique line of cilia under the stigmal vein. The marginal setae 0.03 mm is as long as those on tornus (Plate III, Fig. 1). Genitalia 0.12 mm long, 0.05 mm wide. Triangular dorsal expansion of gonobase has very prominent lobes on each side. Median ventral projection very distinct below the level of the chelate structures (Plate VI, Fig. 1). Apodemes of penis valve is nearly as long as the aedeagus 0.09 mm long. Aedeagus with apodemes of penis valve is shorter than the hind tibia 0.15 mm long (Plate V, Fig. 1).

PLATE I

1 2

4 3

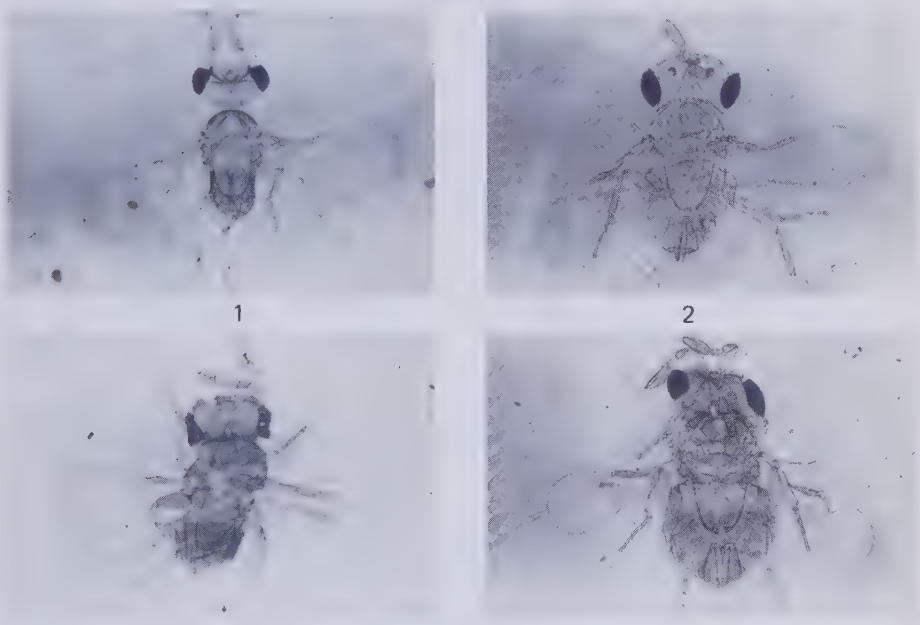
Adult Trichogramma: 1. Adult male *T. australicum* 79x; 2. Adult female *T. australicum* 79x; 3. Adult male *T. chilotraea* 79x; 4. Adult female *T. chilotraea* 79x.

Adult female 0.56 mm long, 0.23 mm wide (Plate I, Fig. 2). Yellow in color with 3 terga abdomen dark pigmented (Plate I, Fig 1). Ovipositor 0.18 mm long, 0.11 mm wide is 0.01 mm longer than the hind tibia (Plate V, Fig. 1). Antennae typically clubbed with short and few hairs.

It was observed that in the field, the efficiency of parasitism was 23.5% on the eggs of *Chilotraea infuscatellus* and 3% on eggs of *Tetramoera schistaceana.*

2. *Trichogramma chilotraea.* Adult male 0.41 mm long, 0.22 mm wide. Yellow in colour with pronotum, mesopluerae, mesoscutum, abdominal terga and hind corae pigmented. Antennae slender tapering hairs ranges from 35-38, with unsegmented antennal flagellum (Plate I, Fig. 2). Forewing 0.43 mm long, 0.25 mm wide with short oblique line of cilia under the stigmal vein. Marginal setae 0.03 mm is as long as those on the tornus (Plate III, Fig. 2). Genitalia 0.12 mm long, 0.25 mm wide with dorsal expansion of gonobase more or less triangular with less conspicuous lobes and tapering apex extending to level of chelate structures which is far below level tips of gonoforcep (Plate VI, Fig. 2). Median ventral projection very distinct and long. Apodemes of penis valve 0.02 mm is shorter than the aedeagus with total length of 0.1 mm. Aedeagus slightly shorter than the hind tibia 0.13 mm long (Plate V, Fig. 2).

PLATE II



Adult Trichogrammatoidea: 1. Adult male *Trichogrammatoidea nana* 79x; 2. Adult female *T. nana* 79x; 3. Adult male *Trichogrammatoidea* sp. 79x; 4. Adult female *T.* sp. 79x.

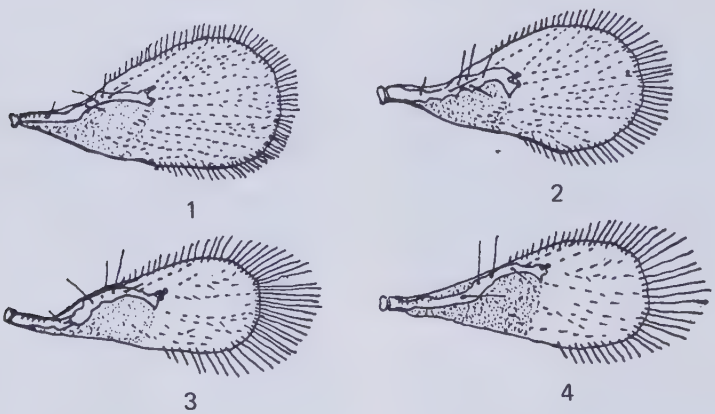
Adult female 0.42 mm long, 0.2 mm wide (Plate I, Fig 4). The colour resembles the male. Ovipositor 0.15 mm long, 0.09 mm wide slightly longer than the hind tibia (Plate V, Fig. 2). Antennae typically clubbed with short and few hairs.

In the field, the efficiency of parasitism on eggs of *Tetramoera schistaceana* was 1% and 76.5 on *Chilotraea infuscatellus.*

3. *Trichogrammatoidea nana* Zehnt. Adult male 0.46 mm long, 0.23 mm wide. Chrome yellow in colour with slight pigmentation of three abdominal terga

(Plate II, Fig. 1). Antennae slender tapering hairs ranges from 21-25 with five segmented antennal falgellum (Plate IV, Fig. 3). Forewing is narrow 0.39 mm long, 0.24 mm wide with the absence of short oblique line of cilia under the stigmal vein. Setae on wing remigium sparse and irregularly arranged. Marginal setae 0.07 mm are twice as long as those on tornus (Plate III, Fig. 3). Genitalia 0.1 mm long, 0.04 mm wide is devoid of dorsal expansion of gonobase. Median ventral projection is prominent below level of chelate structures with medium chitinized ridge extending along the length of fenitalia. Apodemes of penis valve is shorter than aedeagus 0.06 mm long and twice shorter the hind tibia 0.13 mm long.

PLATE III



Forewing: 1. *Trichogramma australicum* 114x; 2. *T. chilotraea* 125x; 3. *Trichogrammatoidea nana* 144x; 4. *T.* sp. 141x.

Adult female 0.46 mm long. 0.24 mm wide (Plate II, Fig. 4). Yellow in colour. Antennae clubbed with few short hairs. Ovipositor 0.12 mm long, 0.09 mm wide slightly shorter than the hind tibia (Plate V, Fig. 3).

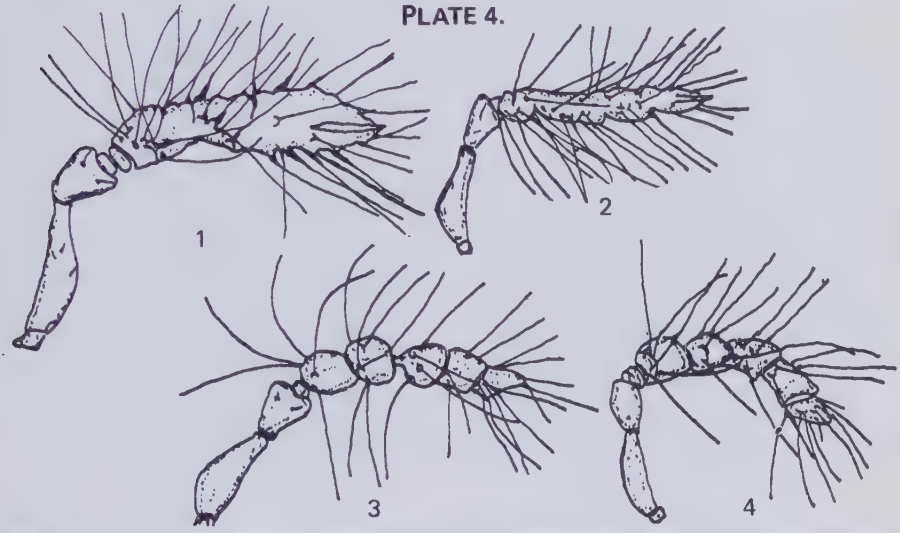
In the field, it was observed that the efficiency of parasitism is specific on eggs *Tetramoera schistaceana* at 91%.

4. *Trichogrammatoidea* sp. Adult male 0.43 mm long, 0.21 mm wide black in colour with dark pigmentation on the anterior portion of the wing extending to the stigmal vein (Plate IV, Fig. 3). Antennae slender tapering hairs ranges from 23-25 with five segmented antennal flagellum (Plate II, Fig. 4). Forewing is narrow, 0.38 mm long, 0.23 mm wide with marginal setae of 0.07 mm which are twice as long as those on the tornus. Short oblique line of cilia under the stigmal vein is absent. Setae on wing remigium sparse and irregularly arranged (Plate III, Fig. 4). Genitalia 0.09 mm long, 0.04 mm wide has no dorsal expansion of fonobase and median ventral projection. Chelate structures near below level of gonoforcep

(Plate VI, Fig. 4). Apodemes of penis valve slightly shorter than aedeagus 0.06 mm long, twice shorter than the hind tibia 0.12 mm (Plate V, Fig. 4).

Adult female 0.49 mm long, 0.24 mm wide resemble the colour of the male (Plate II, Fig. 4). Antennae typically clubbed with few short hairs. Ovipositor 0.13 mm long, 0.1 mm wide slightly shorter than the length of hind tibia (Plate V, Fig. 4).

PLATE 4.



Male antenna: 1. *T. australicum* 291x; 2. *T. chilotraea* 225x; 3. *T. nana* 268x; 4. *T.* sp. 252x.

Similar to *Trichogrammatoidea nana,* parasitism is specific on eggs of *Tetramoera schistaceana* with 5 percent parasitism.

DISTINGUISHING CHARACTERISTICS OF GENERA TRICHOGRAMMA AND TRICHOGRAMMATOIDEA

TABLE 1. List of some distinguishing characteristics that differentiate genera *Trichogramma* from *Trichogrammatoidea.*

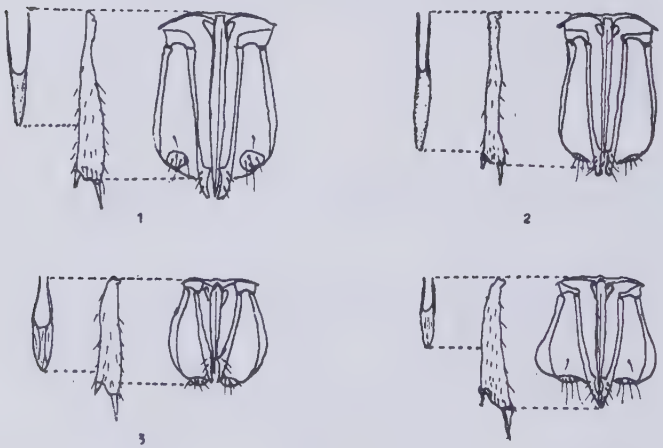
	CHARACTERS	*TRICHOGRAMMA*	*TRICHOGRAMMATOIDEA*
1.	Antenna setae	33 - 38	21-25
2.	Male antennal flagellum	Unsegmented	Five segmented
3.	Arrangement of discal cilia on wing remigium	Plenty closely arranged in distinct rows	Aparse and irregularly arranged
4.	Length of forewing excluding costal marginal setae	0.41 mm - 0.48 mm	0.37 mm - 0.38 mm

5.	Length of marginal setae	0.03 mm	0.07 mm
6.	Length of hind tibia	0.13 mm - 0.16 mm	0.12 mm 0.13 mm
7.	Length of ovipositor	0.15 mm - 0.18 mm	0.12 mm - 0.13 mm
8.	Length of genitalia	0.12 mm	0.09 mm
9.	Length of aedeagus	0.09 mm	0.04 mm - 0.06 mm
10.	Oblique line of cilia	Present	Wanting
11.	Dorsal expansion of gonobase	Present	Wanting

Host Preference

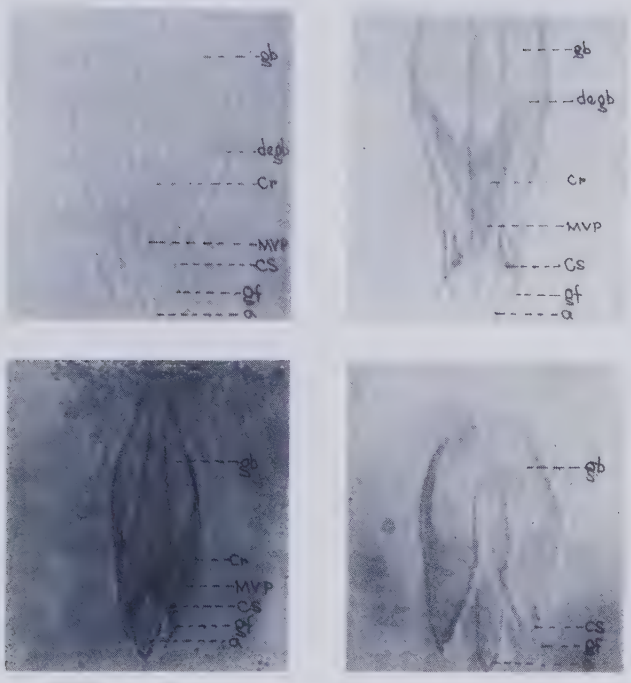
Trichogrammatoidea nana, Trichogrammatoidea sp Trichogramm australicum, and *Trichogramma chilotraea* were found parasitizing eggs of sugarcane stem borers, *Tetramoera schistaceana* and *Chilotraea infuscatellus* in sugarcane fields. In the laboratory *Trichogrammatoidea nana* showed host specificity. It could only be mass reared on *Tetramoera schistaceana.* The last three egg parasites were observed efficient in parasitizing eggs of *Corcyra cephalonica, Tetramoera schistaceana,* and *Chilotraea infuscatellus.*

PLATE 5.



Relative lengths of aedeagus (including apodemes of penis valve), hind tibia and ovipositor: 1. *T. australicum* aedeagus with apodemes of penis valve 378x, hind tibia 313x, ovipositor 300x; 2. *T. chilotraea* aedeagus with apodemes of penis valve 400x, hind tibia 338x, ovipositor 307x; 3. *T. nana* aedeagus of penis valve 450x, hind tibia 246x, ovipositor 250x; I. sp. aedeagus with apodemes of penis valve 330x, hind tibia 308x, ovipositor 285x.

PLATE 6.



Male genitalia: 1. *Trichogramma australicum* 500x; 2. *T. chilotraea* 500x; 3. *T. nana* 500x; 4. *T.* sp. 500x.

a - aedeagus; cr - chitinized ridge; cs - chelate structure; degb - dorsal expansion of gonobase; gf - gonoforceps; mvp - media ventral projection; gb - gonobase.

CONCLUSION

On months where the sugarcane stem borer, *Tetramoera schistaceana* was predominant in sugarcane fields, parasitized eggs collected yielded 91 percent *Trichogrammatoidea nana,* 5 percent *Trichogrammatoidea* sp., 3 percent *Trichogramma australicum,* and 1 percent *Trichogramma chilotraea.* During the abundance of *Chilotraea infuscatellus* parasitized eggs were observed to yield only 76.5 percent of *Trichogramma chilotraea* and 23.5 percent *Trichogramma australicum.*

Trichogramma australicum, Trichogramma chilotraea, and *Trichogrammatoidea* sp. have potential as biological control agents against sugarcane stem borers because the parasites could easily be mass reared on the eggs of *Corcyra* in the laboratory. *Trichogrammatoidea nana* was observed efficiently parasitizing eggs of *Tetramoera schistaceana* and could be mass reared in the laboratory only with the use of *Tetramoera schistaceana* eggs.

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UN AGENTE PROMETEDOR ENCONTRADO EN LA ISLA NEGROS EN FILIPINAS PARA EL CONTROL BIOLOGICO DE LOS BARRENADORES DE LA CAÑA DE AZUCAR

M. C. Alba y B. R. Estioko

RESUMEN

Se describen cuatro especies de *Trichogrammatidae* que habitan en la Isla Negros de Filipinas. Dos especies son del genero *Trichogramma* y otras dos del genero *Trichogrammatoidea.* Estas cuatro especies de *Trichogrammatidae* son agentes prometedores contra los barrenadores *Tetramoera schistaceana* Sn. y *Chilotraea infuscatellus* Sn. Se pueden criar en el laboratorio *Trichogramma australianum* Girault, *T. chilotraea y Trichogrammatoidea* sp. sobre huevecillos de *Corcyra cephalonica* Stainton, mientras que *Trichogrammatoidea nana* Zehnt. solo se puede criar sobre huevecillos de *Tetramoera schistaceana* Sn. tanto en el laboratorio como en el campo. Observaciones de campo indicaron que el parasito mas efectivo contra el huevecillo de *C. infuscatellus* es T. chilotraea, con un parasitismo de 76.5 porciento, y *T. nana* contra *T schistaceana* con un parasitismo de 91 porciento.

BIOLOGICAL AND ECOLOGICAL FACTORS AFFECTING THE CONTROL OF SUGARCANE BORER (*DIATRAEA SACCHARALIS* F.) IN BARBADOS, W. I.

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ABSTRACT

Several ecological changes such as pre-harvest burning, and change and predominance of certain varieties occurred from the introduction and establishment of two larval parasites of the sugarcane borer *Diatraea saccharalis* (F.). This led to substantial reductions in the level of joint infestations and sugar loss in Barbados.

Parasites reduced joint infestation by about 8%; pre-harvest burning reduced joint infestation in the presence of the parasite complex by a further 4%; and the change to a more vulnerable variety increased the average joint infestation by perhaps 1-11/2%.

INTRODUCTION

Prior to 1966, the joint infestation of *Diatraea saccharalis* (F.) in Barbados has averaged 15.5% over several years. Subsequently, with the introduction and establishment of two larval parasites, *Apanteles flavipes* (Cam.) and *Lixophaga diatraeae* (Tns.), the joint infestation fell sharply, reaching a low of 1.9% in 1974, and since then it has risen again. Concurrently, there was also a fundamental change in the cane field ecology brought about by the increased practice of pre-harvest burning. This practice declined sharply in 1974, but began to increase again in the following years.

The present study records and analyses the pest population in relation to parasite introduction, fire, and varietal changes, and the fluctuations, and possible interactions of the two parasites mainly contributing to the control of the pest.

RESULTS AND DISCUSSIONS

Joint Infestation

Fig. 1 shows the levels of total percent parasitism by *Apanteles* and *Lixophaga,* 1968-78. The contribution of each species to the total parasitism is also given.

Joint infestation assessment was based on the same method throughout (Metcalfe[4])

Parasites

Reduction in joint infestation since 1966 can be attributed to the introduction and establishment of the two larval parasites, *A. flavipes* and *L. diatraeae.* Details of this establishment have been given by Alam *et al*[1]. *Apanteles* was recovered one year after its first release in 1966, and then built up rapidly. The population was augmented by additional releases during 1967-71. *Lixophaga* was unsuccessfully introduced during the 1930 and 1940s. A massive campaign initiated by Metcalfe during 1958 and continued by Van Whervin until 1963, when over 1/2 million adults were released, resulted in the establishment of the parasite, but the population remained very low. From 1978 onwards, although the releases made were very small, the parasite gradually built up its population and spread throughout the island.

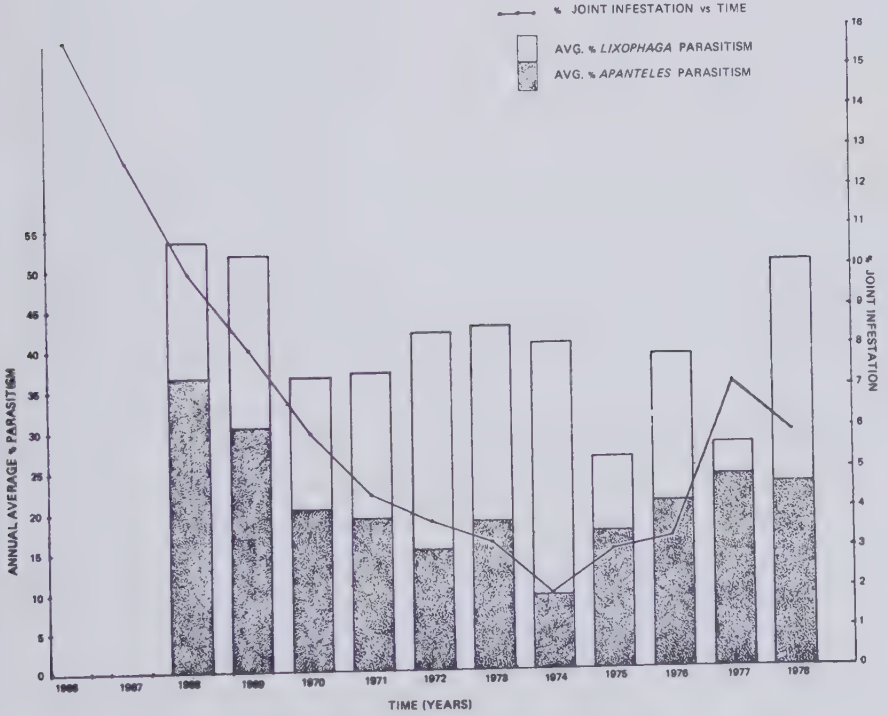

FIGURE 1. Level of joint infestation, in relation to parasitism by *Apanteles flavipes* (Cam.) and *Lixophaga diatraeae* (Tns.), from 1966 to 1978, in Barbados.

Burning

Before 1965, burning was not a common practice in Barbados, but from then onwards there was an increase in pre-harvest burning. Table 1 gives the percent acreage burnt from 1965-78.

TABLE 1. Percentage acreage of canes burnt in Barbados between 1965 and 1978.

Year	Cane burnt (% of total acreage)	Year	Cane burnt (% of total acreage)
1965	6	1972	86
1966	11	1973	85
1967	22	1974	85
1968	33	1975	8
1969	30	1976	3
1970	40	1977	15
1971	60	1978	19

In Fig. 2 the level of joint infestation and the average annual total larval parasitism by *Apanteles* and *Lixophaga* is super-imposed on a graph of acreage burnt. It appears that although the overall annual parasitism by the two parasites remained fairly high, there was an inverse relationship between burning and joint infestation: as the burnt cane increased, the level of joint infestation dropped, while with the reduction in burning, borer damage increased to about 7%, and again fell to 5.9% when the acreage burnt was increased.

Parasites/Fire/Joint Infestation

It is interesting to try and aportion the total affect of burning and parasitism on the general reduction of the pest as follows:

A. The typical level of pest infestation before burning or introduction of parasites was around 15%;

B. The trend level with burning + parasites seemed to be around 3%.

C. The level of joint infestation after burning stopped, with parasites remaining as the major controlling factor appears to be around 7%.

Therefore the effect of parasites alone is A - C, i.e. about 8% reduction, and the effect of fire alone is C - B, i.e. about 4% reduction.

That burning might have an appreciable effect in reducing the pest population is contrary to earlier opinion in Barbados. For example work reported by Alam[1]

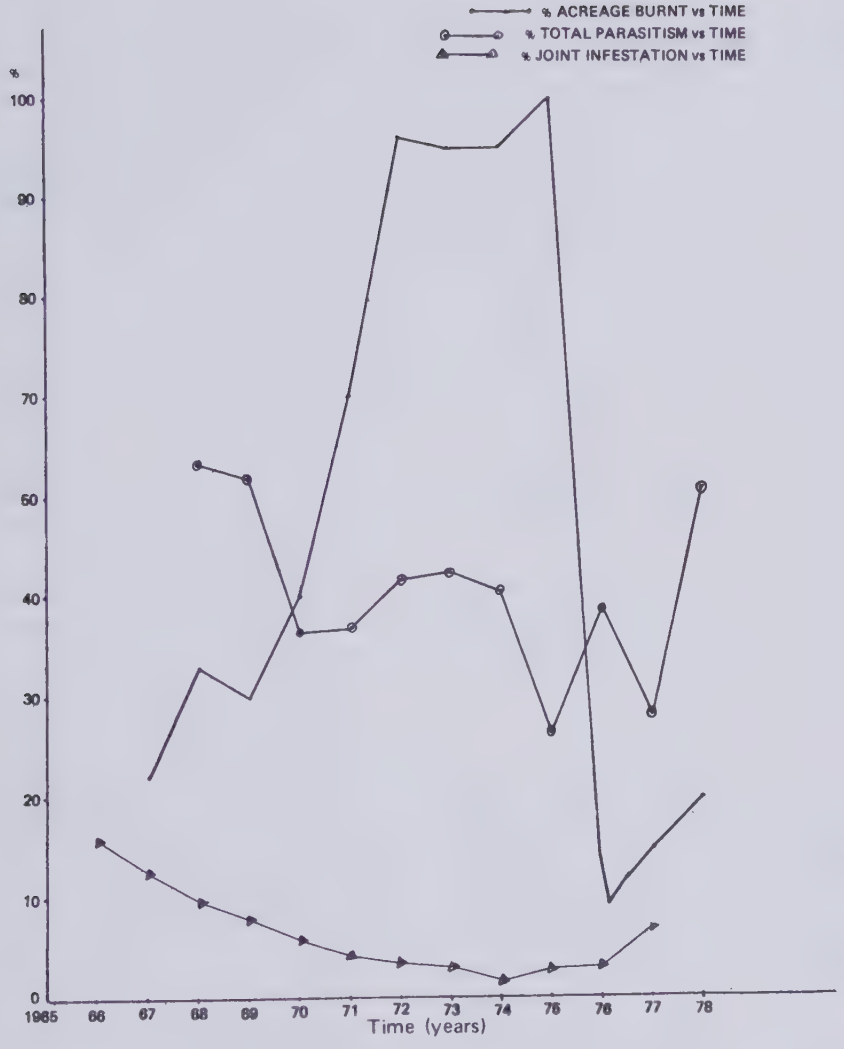

FIGURE 2. Effect of sugarcane burning on the levels of parasitism by *Apanteles flavipes* (Cam.) and *Lixophaga diatraeae* (Tns.) and on joint infestation, between 1966 and 1978.

involving neighboring burnt/unburnt situations, showed that burnt sugarcane fields are preferred for reinfestation by *Diatraea*. In fact such fields initially have exceptionally high pest populations (Table 2). But even in burnt fields, where the initial parasitism was nil to very low, there was a rapid build-up of the parasites in a few months (Alam[1]). If further work confirms that fire did indeed exert such a control in the 1970-74 period then this would be an interesting example of the danger of extrapolating from good, but localized, experimental data to a larger scale.

TABLE 2. Percentage dead hearts recorded in burnt and unburnt sugarcane fields In Barbados, for the first four months after harvest.

Fields No.	Variety	Average % Infestation Burnt	Unburnt
1	B-62163	41.6	5.5
2	"	36.6	5.2
3	"	20.1	7.2
4	"	47.7	6.7
5	"	64.8	6.5
6	" "	61.7	10.2
7	"	39.0	5.0
8	B-70574	42.3	4.0

While burning is one interpretation of the reduction in infestation levels, there are other possible explanations, including variety changes.

Varietal Susceptibility

The borer infestation varies considerably between different varieties of sugar cane. This could be because of the differential attraction of the host plants to gravid females, favorable or unfavorable characters (e.g. rind hardness) of the plants for penetration, and the establishment and development of the borer larvae. These together determine the ability of the plants to resist borer attack. Tucker[8,9], in Barbados, and Henseley and Long[3] in Louisiana reported the differential susceptibility of various sugarcane varieties to the borer.

From 1972 onwards, variety B-62163 rapidly replaced the other varieties, and at present it occupies over 60% of the area under sugarcane (Table 3).

This variety is high yielding and of wide ecological adaptation. However, it has been found to be more susceptible to such pests as the sugarcane borer. (Table 4).

Reviewing the data in Tables 3 and 4 and in Fig 2, the percentage increase and the timing of that increase in variety B-62163 cannot fully account for the fluctuations in infestation. However, it could clearly be a contributing factor (perhaps to the extent of about 1.0 - 1.5%) in the increase in joint infestation.

Parasite Interaction

The introduction of more than one parasite species attacking the same host stage may result, particularly when parasitism is high, in direct competition for food between parasite larvae inside the host. Subsequently, the most aggressive parasite survives, while the other is usually suppressed or eliminated. With this in mind, before the introduction of natural enemies into a new area, it is important to consider whether to introduce several parasite species, which may compete with each other, or to introduce only one species of natural enemy.

Smith[7] analyzed the problem of multiple parasitism, and refuted the claims of Pemberton and Willard[6], who stated that *Opius humilis* Silv. acting alone would have caused greater mortality of *Ceratitis capitata* (Wied.) than the actual mortality caused by all the introduced fruitfly parasites. This was because the larvae of

TABLE 3: Percentage acres sugarcane planted of the more important varieties in Barbados.

Year	B 4744	B 49119	B 59162	B 60267	B 62163
1967	27.21	27.68		–	
1968	29.22	26.50	–	–	–
1969	31.08	22.11	–	0.62	–
1970	32.62	18.49	–	1.82	
1971	32.73	12.62	1.53	5.40	–
1972	32.12	7.25	7.31	10.47	3.46
1973	25.19	4.56	14.55	11.31	13.01
1974	18.90	2.32	18.15	12.18	25.28
1975	13.47	0.48	19.16	11.34	39.67
1976	7.70	0.81	15.97	8.61	52.48
1977	4.04	0.50	13.01	6.14	59.25
1978	2.58	0.34	8.53	5.23	60.17

TABLE 4. Joint infestation level in four different varieties recorded during 1978 and 1979 in Barbados.

Year	B–62163			B–59162			B–63118			B–60267		
	Tops	Bottoms	Total	Tops	Bottoms	Total	Tosp	Bottoms	Total	Tops	Bottoms	Total
1978	7.9	4.4	6.2	3.4	1.4	2.5	5.9	3.3	4.6	5.3	3.7	4.5
1979	6.7	4.7	5.7	3.3	3.4	3.4	4.8	3.7	4.3	2.7	1.1	1.9
Ave.			5.9			2.9			4.4			3.2

Opius tryoni (Cam.) and also of other parasites, always eliminated the larvae of *O. humilis* Smith [7] concluded that multiple parasitism never resulted in a lower total mortality of the host than would have occurred if one or other parasitic species had been acting alone.

Fig. 3 shows the relative abundance of the two parasite species over the growing/harvesting period. The date for each month is the average of 10 years data.

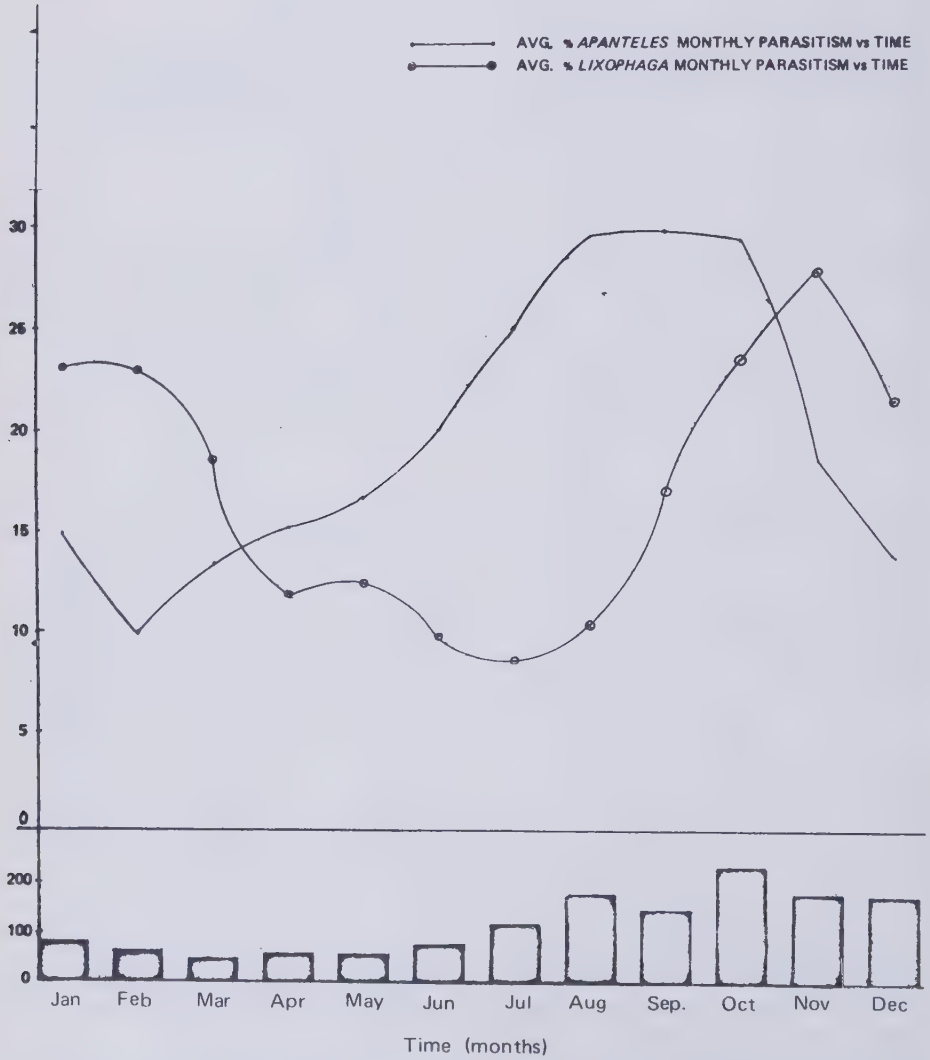

FIGURE 3. Relative abundance of the two parasites, *Apanteles flavipes* (Cam.) and *Lixophaga diatraeae* (Tns.) and the rainfall pattern over the growing/harvesting period.

Table 5 gives the results of competition tests between *Apanteles* and *Lixophaga*. The column "Intervals in days" represents the time between stinging of *Diatraea larvae* by *Apanteles* and subsequent inoculation of the parasitised larvae by *Lixophaga* maggots.

After its establishment in Barbados, *Apanteles* increased rapidly, and soon outnumbered *Lixophaga*. From then until 1974, the population of *Apanteles* declined but built up again between 1975-78 (concurrent with the increase in the level of joint infestation). Initially this reduction in the population of *Apanteles* was considered probably due to the intrinsic superiority of *Lixophaga*. But this may not necessarily be true for the following reasons (i) The shorter life cycle of *Apanteles;* (ii) the fact that soon after emergence, *Apanteles* adults mate and immediately attack available host larvae, in contrast to *Lixophaga* females, which after mating, have to complete a gestation period of 7 to 9 days before they can deposit their progeny near the entry holes of *Diatraea* larvae; (iii) *Apanteles* has a greater searching capacity than *Lixophaga*, as the former first locates the host larva and attacks it, without any wastage of its progeny, whereas the latter has the additional hazard involved in that its larvae are deposited at the tunnel entrances, and have to search out their host inside.

TABLE 5: Inter-competition tests between *Apanteles* and *Lixophaga* (number of Larvae stung by *Apanteles* on day 0 and subsequently inoculated by *Lixophaga* maggots, 1 to 5 days later).

Interval *in* days	No. *Diatraea* larvae stung & inoculated	Percent parasitism *Apanteles* No. larvae parasitized	% parasitized	*Lixophaga* No. larvae parasitized	% parasitized	Both
1	191	6	3.14	185	96.86	Nil
2	187	3	1.60	184	98.40	Nil
3	240	9	3.75	231	96.25	Nil
4	95	12	20.95	83	79.05	10
5	69	37	59.49	32	40.51	10

With regard to parasite competition Table 5 shows the following: Where the larvae were parasitized both by *Apanteles* and *Lixophaga,* the latter has a definite advantage even where the former has 3 days start in larval development. With an increasing interval in days, there is a greater chance of survival of both, perhaps even a possibility of some advantage to *Apanteles.*

It is also likely that certain weather conditions, or factors like cane height, favor one parasite, while other situations favor the other parasite.

In the field (Fig. 3) the population trends of *Apanteles* followed the level of joint infestation levels quite closely suggesting a strong tendency towards pest population dependence, whereas *Lixophaga* appears to be free from this dependence, as the fluctuations in its population from year to year cannot be correlated with joint infestation levels. However, the period of lowest joint infestation does appear to be somewhat associated with above average proportions of *Lixophaga* parasitism.

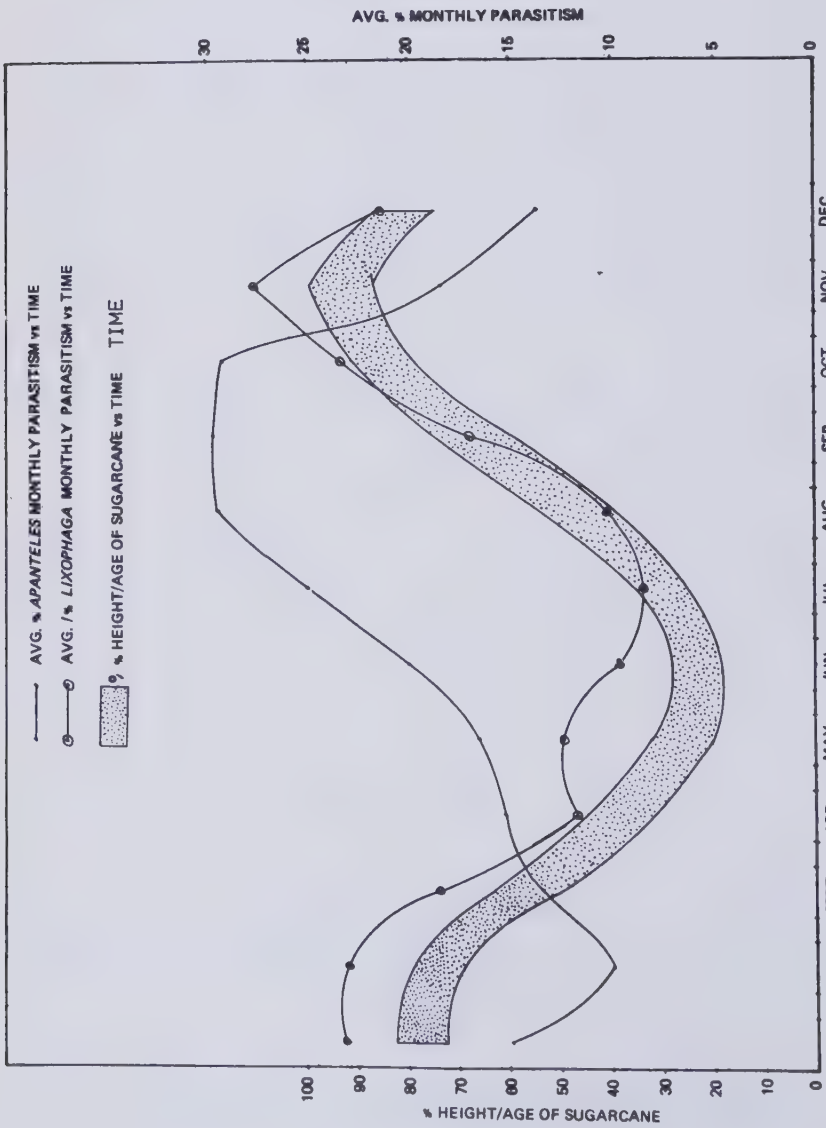

FIGURE 4. Relative abundance of *Apanteles* and *Lixophaga* in relation to the height/age of sugarcane.

As to seasonal variations, *Lixophaga* increases in spite of a high population of *Apanteles,* whereas *Apanteles* increase is concurrent with a decrease in *Lixophaga.*

It is further suggested that the population fluctuation of *Lixophaga* is related to the height of the sugarcane plant. The model given in Fig.4, attempts to depict the general height (age) of sugarcane plants in the island over a 12-month period. The sharp fall in the height estimate in November is due to newly planted cane fields becoming available for searching by the field team assessing *Diatraea* damage. This model appears to coincide closely with the fluctuations of *Lixophaga* populations as if the taller canes favour the parasite.

As conditions increasingly favour *Lixophaga,* its competition with *Apanteles* increases, and so in the period of tallest (mature) canes, one sees a higher percentage of parasitism due to *Lixophaga,* while in shorter (young) canes, the level of parasitism is due mainly to *Apanteles.*

On the whole, the data seems to support Smith's contention that two parasites, even though naturally competitive, result in a high total parasitism. In Fig. 1, total parasitism seldom fell below 35% in spite of quite wide variations in the populations of the individual parasites.

CONCLUSIONS

There has been a reduction in the level of infestation in sugarcane by the sugarcane borer since 1966, attributed mainly to two larval parasites, *Apanteles flavipes* (Cam.) and *Lixophaga diatraeae* (Tns.), which were introduced and became established in the island. Several other factors, however, show some correlations with the level of joint infestation. In particular, the increase in acreage burnt prior to harvest appears to have been associated with a reduction in joint infestation, and the widespread use of B-62163 may be associated with an increased level of joint infestation.

The evidence indicates that the two parasites have brought about a reduction of about 8% in joint infestation. At this degree of control, there is evidence that *Lixophaga* limits population of *Apanteles,* the latter rising in numbers only when the *Lixophaga* is disadvantaged by the general lack of cover in the fields.

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FACTORES BIOLOGICOS Y ECOLOGICOS QUE INFLUYEN EN EL CONTROL DEL BARRENADOR DE LA CAÑA DE AZUCAR *DIATRAEA SACCHARALIS* (F.) EN BARBADOS, W. I.

M. M. Alam

RESUMEN

Desde la introduccion y el establecimiento en la isla de Barbados de 2 parasitos arvales del barrenador de la caña de azucar (*Diatraea saccharalis* (F.) que redujeron considerablemente el indice de infes-

tacion y las perdidas en sacarosa, se han registrado ciertos cambios ecologicos en la Isla. Como por ejemplo, la quema de caña antes de la cosecha, incremento de nuevas variedades, etc.

Se deduce que los parasitos redujeron en un 8 porciento el indice de infestacion, la quema antes de la cosecha en presencia de dichos parasitos en un 4 porciento adicional. Pero el cambio de variedades aumento dicho indice de infestacion en un 1 a un 1-1/2 porciento debido al incremento en variedades mas vulnerables a los ataques del barrenador.

THE BIOLOGY OF SOFT SCALE INSECT *PULVINARIA ICERYI* (SIGN.)

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ABSTRACT

The salient features of the biology of *Pulvinaria iceryi* (Sign) are described and 25 known host plants are listed. The insect can drastically affect sugarcane by withdrawing phloem sap and possibly by a toxic action that follows feeding. It is considered a dangerous insect wherever it coexists with sugarcane .

INTRODUCTION

In 1862-64, the soft scale *Pulvinaria iceryi* (Signoret) (Coccidae, Homoptera) (Fig. 1), then an undescribed species, devastated the sugarcane plantations of Reunion and Mauritius (Icery[16]). After the outbreak, the insect became scarce and the coccidologist De Charmoy[7,8,9] searched for it in vain in Mauritius for many years at the beginning of this century. Subsequently, in Mauritius, the insect was occasionally found on sugarcane in the greenhouse (De Charmoy[10], Mouetia[19]) and a small localized field infestation occurred in 1955 (Mamet[17]) and in 1959 (Williams[22]).

In 1975-77 an outbreak of the insect occurred in both Reunion and Mauritius, and in the latter the dramatic events of 1862-64 were repeated. Scattered, small infestation in sugarcane were seen first in Reunion in 1975 and then in Mauritius in 1976. The infestations remained very localized in Reunion and receded by the end of 1976 without having caused any significant economic loss (Ettiene[11]). In Mauritius, on the other hand, foci of infestation, first noted in June 1976, became widespread in the later months of that year and many began to expand rapidly. By March-April 1977, the outbreak had reached alarming proportions: intense populations of the insect had utterly destroyed many fields of cane and severely damaged others. About 4200 ha (5% of the island's total cane area) were affected to some extent at this period. During May, it became apparent that the outbreak had passed its peak and, hastened by early cropping of infested fields, the insect's populations declined rapidly until only a few limited colonies remained at the end of the year. These eventually disappeared and in 1978 the insect caused no loss and was confined to a few small pathches of cane here and there.

The Mauritian sugar industry lost 15-20,000 tons of sugar in 1977 from the attacks of *P. iceryi* (Anonymous[3]). This represents 2-3% of a "normal" annual production of 700,000 tons. At world sugar prices in 1977, this was the equivalent of about Rs 18-24 million (£ 1.5-2.0 million). In addition, the cost of replanting destroyed fields and recruiting (filling the gaps) in other attached fields has been estimated at about Rs 27.5 million (£ 2.3 million).

Identity, Geographical Distribution and Related Species

P. iceryi is authentically recorded from Mauritius, Reunion, Agalega, South Africa, Rhodesia (Mamet[17]), Madagascar (Mamet[17]), Zambia and Uganda (D. J. Williams, Commonw. Inst. Ent. *in litt.*), indicating that it exists throughout eastern and southern Africa and has been known to damage sugarcane only in the Mascarenes. Its occasional presence on sugarcane in the greenhouse has been reported in Natal (A. J. M. Carnegie, S. Afr. Sug. Ass. Exp. Sta., *in litt.*), while a species stated to resemble *P. iceryi* is recorded from sugarcane in the field in Mozambique (Goncalvez[13]).

Other species of *Pulvinaria* reported on sugarcane are *P. elongata* Newstead in the Western Hemisphere and *P. saccharina* De Lotto in Natal[20]. One or more species of uncertain identity (although recorded as *P. iceryi*) occur on sugarcane in New Guinea and China[20]

The present study aimed to describe the salient features of the insects biology, its natural enemies and its damage to sugarcane.

METHODS AND DISCUSSION

Life Cycle and Habits

The life stages and development

Males do not exist and reproduction is parthenogenetic and oviparous. The eggs are spindle-shaped, about 340 μm long by 160 um wide, and have a faintly roughened chorion. Almost colorless and translucent when freshly-laid, they become amber-yellow later.

The number of female larval instars in *Coccidae* (s.s) is given by various authors (Annecke[1], Canard[5,6], Husseiny and Madsen[15]) as two or three. There are three in *P. iceryi.* The larval instars and the young adult are of similar form and the separation of one from the other is not possible with the naked eye, particularly as body size increases greatly within each instar. The most convenient character for identification of instar is the number of marginal (lateral) setae along any particu-

lar portion of the body margin, e.q. between the eyes, or anterior to the first pair of spiracles (Table 1). These can be counted with a x 10 hand lens on living individuals. With slide-mounted specimens, the size of the leg segments also identifies instar (Table 1). The first instar is further characterized, as in other Coccoidea, by two very long posterior setae that are lost at the first molt. The mature adult is identified by its ovisac, and its maximum attainable length, excluding ovisac, is about 4.5 mm.

Secretion of the ovisac under the posterior end of the body is continuous throughout the oviposition period and as the ovisac increases in size the hind end of the female's body is levered away from the plant surface (Fig 1). The eggs are laid into the cavity of the ovisac where they are enmeshed in a loose tangle of waxy threads. Hatching occurs in the ovisac and the young larvae escape by pushing their way through the strands of the felt-like ovisac wall, which is of irregular thickness and density and often splits lengthwise from pressure of eggs accumulating within.

The newly-hatched larva (the "crawler") is the only habitually mobile stage and is therefore the true infective stage. However, the subsequent stages are all capable of movement, albeit cumbersome, until secretion of the ovisac begins. This structure, being firmly stuck to the plant surface, precludes further possibility of movement. The retention of the power of movement by post-crawler stages appears to be general in Coccidae (Annecke[1], Bodenheimer[4]; Canard[5,6]) but is a survival rather than a dispersal mechanism. Thus, in *P. iceryi,* individuals often move for no apparent reason after settling on the host-plant, shifting their position slightly or considerably, but the tendency is to stay in one place, or at least in the immediate vicinity of the spot where development began, and there is no general movement unless the plant tissues on which feeding is occurring begin to dry out. If this happens, there is a migration to new feeding sites and the only individuals to remain are those incapacitated by parasitism and adults with ovisacs. During move-

TABLE 1. Characters distinguishing the instars of *Pulvinaria iceryi*

	No. prespiracular marginal setae				Length of anterior femur-trochanter (μm)*			
Instar	Mean	S. D.	Range	*n*	Mean	S. D.	Range	*n*
1	13.5	0.6	12-14	29	74	3	70 -82	28
2	34.3	2.8	29-40	44	99	6	86 -110	43
3	54.4	3.8	46-61	27	134	7	120-44	27
4	82.4	7.0	71-101	36	198	17	178-235	36
(adult)								

*one measurement per individual

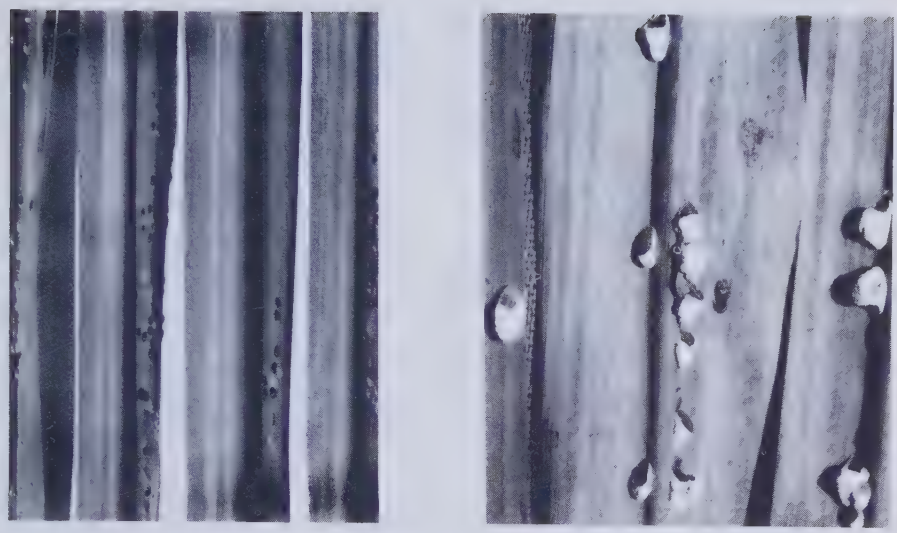

FIGURE 1. *Left:* Adults of *Pulvinaria iceryi* with their conspicuous white ovisacs. *Right:* young stages killed by the hymenopterous parasite *Coccophagus cowperi* (the host dorsum turns black when parasitized by this species).

ment, the stylet is carried in the crumena.

When molting, the exuvium emerges from under the posterior end of the body as a crumpled, drawn-out, greyish mass and eventually falls away. The process is accompanied by contractile movements of the body, while the posterior lobes of the body open and close, pincer-fashion, crushing the exuvium and apparently tugging it backwards. There is no change of position during molting. As noted in some other *Coccidae* (Annecke[1]) the dorsum of the integument (the entire upper surface to near the lateral marginal setae) is not shed but remains *in situ* and fragments during subsequent growth, as may be demonstrated by marking the dorsum before molting with ink, etc. The stylet is not left in the plant tissues, as occurs in *Diaspididae,* (William[23]) but is withdrawn and remains part of the cast-away exuvium.

The feeding site

P. iceryi lives on the photosynthetic tissues of its host-plants, generally on the undersurface of the leaf blades. On sugarcane, it may also occur on the leaf sheath near its junction with the leaf blade, particularly on small shoots. On grass weeds, especially on the less robust species, it has been seen on the stems and on the rachis of the inflorescence. Individuals are always aligned with their long axis more or less parallel with the vascular bundles and they may be head up or head down.

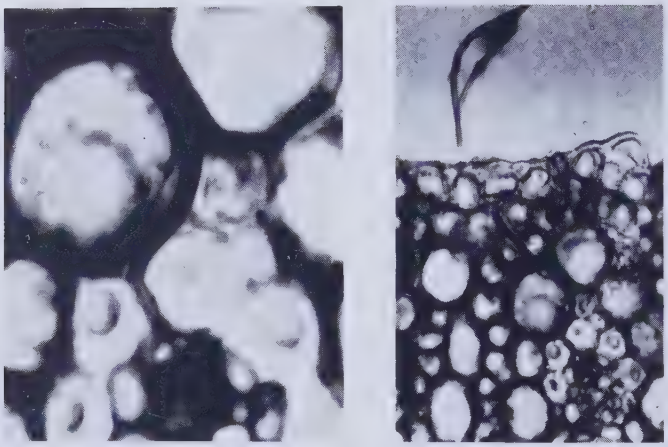

FIGURE 2. Transverse section of sugarcane leaf showing the stylet of *Pulvinaria iceryi* reaching to the phloem. (a) bundle sheath, (b) Phloem fibers, (c) phloem, (d) xylem.

The feeding site within the plant is the phloem and the path of the stylet is intercellular (Fig. 2). The stylet is relatively short, being about 160 μm in the 1st instar and about 280 μm in the adult, and is adapted for the shallow penetration needed to reach the vascular bundles of photosynthetic tissues. By contrast, *Aulacaspis tegalensis* (Diaspididae), which feeds in the paranchyma cells of sugar cane stems, has a stylet 300 μm long in the 1st instar and 2400 μm long in the adult (William[23]).

Duration of life cycle

On sugarcane in the greenhouse at Reduit, the duration of the life cycle from egg laid to initial oviposition varied from 35 to 60 days according to season. The host-plant and its condition also probably affect the duration of life cycle, although this has not been demonstrated. The oviposition period, which begins about a day after secretion of the ovisac starts, may last 20 days or more. Death follows shortly after oviposition stops.

Fecundity

Counts of eggs in the ovisac of nine females on sugarcane selected for the large size and the fresh condition of their ovisacs ranged from 754 to 1257 and averaged 950/ovisac. However, the oviposition period of females on sugarcane can exceed the incubation period by many days so that a count of unhatched eggs in the ovisac at any time underestimates fecundity, which may therefore greatly exceed 1000 eggs/female. The final size of ovisacs, and by implication fecundity, varies. One factor is premature death of infested leaves before completion of the

lengthy oviposition period and the inability of ovisac-bearing females to migrate from a dying leaf to a new feeding site. Condition of the host-plant, in particular the size and vigour of the shoots on which development is occurring, is probably another factor, while according to Etiene[12] fecundity is greater on sugarcane than on the weed *Setaria barbata*. This author recorded 1728 eggs from one female on sugarcane.

Host-plants

P. iceryi has been recorded from 25 named *Gramineae,* or 30 if those seen bearing only immature insects are included (Table 2). During the epidemic conditions in Mauritius in 1976-77, a few ovisac-bearing adults were also found on *Cyperus rotundus, Kyillinga* sp. (both Cyperaceae) and *Bidens pilosa* (Compositae), all growing in or near infested cane fields, but development on such plants was clearly exceptional *P. iceryi* must be considered a grass-inhabiting insect able to develop on many grass species, although no doubt some are more suitable for its development than others. In this respect, it is worth noting that the insect is not recorded on maize and it failed to develop on this plant in a greenhouse test.

Dispersal

As mentioned above, all larval stages and the young adult are ambulatory but the true infective stage is the newly-hatched larva before it begins to feed (the "crawler"). The ability of Coccoid crawlers to disperse unaided is limited by their small size and by their need to settle and feed without undue delay. Dispersal over any considerable distance is therefore passive, eggs and/or crawlers being carried on plant material, field workers, animals, etc. Crawlers are also carried by wind.

There is adequate evidence (Greathead[14]) to conclude that short and long distance dispersal of Coccoidea is at times effected by wind. A simple experiment illustrated how wind assisted short-distance dispersal of *P. iceryi* in the cane fields of Mauritius during the recent outbreak. Fifty 76 x 26 mm microscope slides were smeared on one side with vaseline and laid out at 1m intervals along the middle of a 4m-wide road between two severely infested fields of sugarcane where vegetation was about 2m high. Every alternate slide was raised on a cork standing in a dish of oil, thereby ensuring that all crawlers trapped by the vaseline had been airborne. Exposed from 9:00-12:00 h on a fine but gusty morning, the slides isolated from the ground caught the equivalent of 3175 crawlers $m^{-2}h^{-1}$. The slides in contact with the ground trapped nearly six times as many. Thus, ground in the near vicinity of infested plants had a high crawler population that was being rapidly augmented by airborne individuals.

Mutualism with ants

P. iceryi produces copious amounts of honeydew and is tended by various

TABLE 2. Graminaceous host-plants of *Pulvinaria iceryi*

Country	Host-Plant	Reference/Remarks
South Africa	Indet. grass	17
	Hyparrahenia hirta	2
Rhodesia	Indet. grass	17
Zambia	*Eleusine corocana*	D. J. Williams, CIE, *in litt.*
Uganda	*Digitaria scalarum*	" " "
Madagascar	"hahomosa"	18
Agalega	*Digitaria didactyla*	17
	Panicum maximum	17
Reunion	*Panicum maximum*	12
	Rottboellia exalta	12
	Saccharum officinarium	12
	Setaria barbata	12
	Sorghum verticilliflorum	12
	Thysanoena maxima	12
Mauritius	*Brachiaria reptans*	24
	Cenchrus echinatus	24 (only immature insects seen)
	Cynodon dactylon	17, 24
	Digitaria biformis	24 (only immature insects seen)
	Digitaria fuscescens	24
	Digitaria timorensis	24
	Digitaria (?) borbonica	24 (only immature insects seen)
	Eleusine indica	24
	Panicum maximum	24
	Panicum subalbidum	24 (only immature insects seen)
	Paspalidium geminatum	17
	Paspalum commersoni	24
	Paspalum conjugatum	24
	Paspalum paniculatum	24
	Paspalum urvillei	24
	Pennisetum paniceum	24 (only immature insects seen)
	Pogonatherum panicum	24
	Saccharum officinarum	17, 24
	Setaria barbata	24
	Setaria pallide-fusca	24
	Sporobulos indicus	24
	Stenotaphrum dimidiatum	24
	Vetiveria zizanioides	24

species of ants, among which are *Technomyrmex detorquens* Wlk., *Anoplolepis longipes* Jerd. and *Pheidole* sp. The hygenic action causing them to become sticky and covered with black mold is advantageous to *P. iceryi.*

Natural Enemies

The parasites and predators of *P. iceryi* in Mauritius, and their interactions as far as these are known, are shown in Fig. 3. The Coccinellid predators, in larval and adult stages, eat the ovisacs of *P. iceryi* but show no apparent interest in the immature stages. Of the hymenopterous parasites, two species, *Aneristus cero plastae* and *Coccophagus cowperi,* display sex-linked hyperparasitism, so that the interactions between parasites and hyperparasites are extremely complex.

The combined action of natural enemies on *P. iceryi* in Mauritius in 1976-77 was obviously great. The Coccinellids, of which *Hyperaspis hottentota* and *Exochomus laeviusculus* were the commonest, tended to become important mortality factors after an attack had progressed, when ovisac-bearing *P. iceryi* were abundant enough in a field to retain vagrant adults and provide ample food for concentrated in-field reproduction. They appeared to be of no great value in checking incipient attacks. By contrast, the Aphelinid parasites *A ceroplastae* and *C cowperi* (Fig. 1), and the Encyrtid *Metaphycus* sp., destroy immature *P. iceryi.* The other common parasite, *M. decussatus,* often permits its host to lay many eggs before killing it. The important hyperparasites of these species were *Tetrastichus* sp. in *A. ceroplastae* and *C. cowperi,* and *Silvestria minor* in *M. decussatus.* Other species named in Fig. 3 appeared to be of lesser importance.

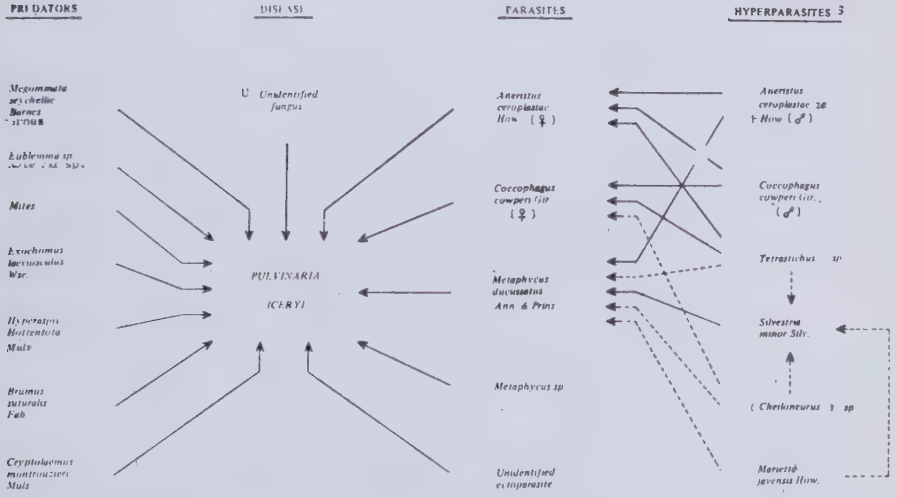

FIGURE 3. Natural enemies of *Pulvinaria iceryi* in Mauritius and their interactions as far as known. *Plain lines:* action definite. Broken lines: action indicated by observations.

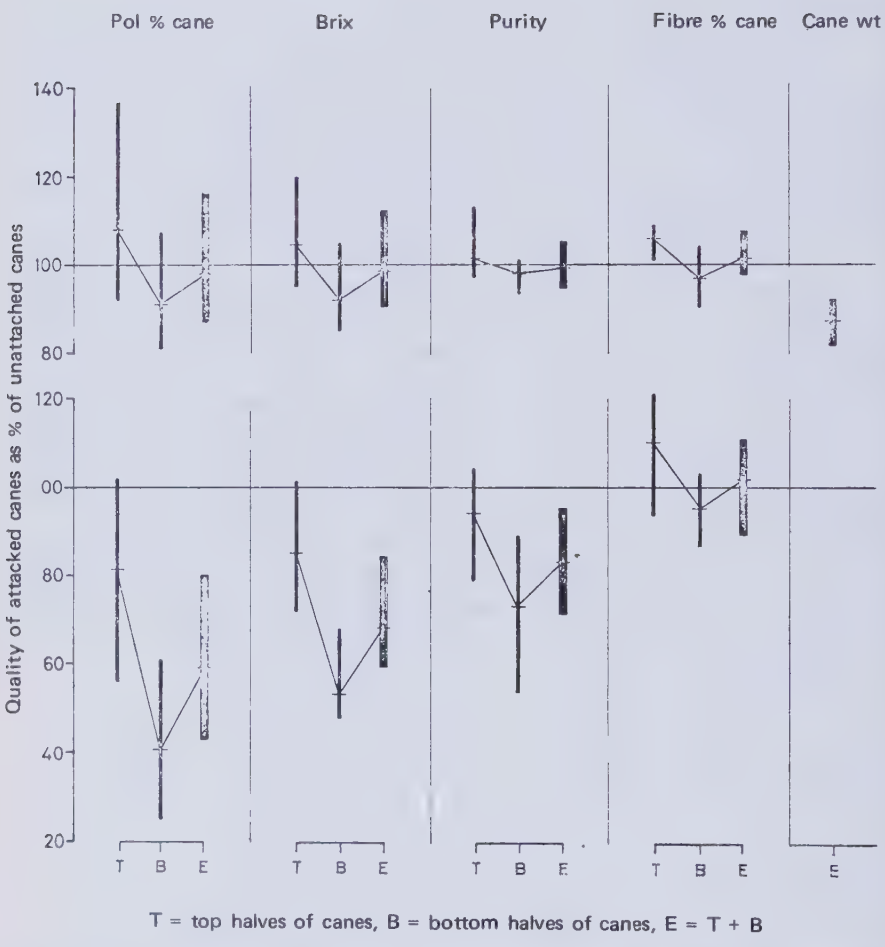

FIGURE 4. Altered composition of millable canes after attack by *Pulvinaria iceryi*. Results from unattacked, slightly attacked, and severely attacked canes taken from six fields of var. S 17, May 1977. *Above:* Slightly attacked, *Below:* Severely attacked. Vertical bars show range, means are linked.

Effects of Infestation in Sugarcane

Infestation in sugarcane causes reduction or stoppage of growth, loss of normal leaf color and premature death of leaves. The outright death of even large plants may result within a few weeks when infestation is severe. Plants that survive attack may remain sickly and die later, or fail to ratoon after cropping. Many entire fields were utterly destroyed in Mauritius in 1977, while greatly reduced cane yields followed by ratooning failure occurred in others.

The action of the insect on the sugarcane plant is relatively drastic and requires clarification by experimental work. There is reason to believe that the insect's harmful effect on sugarcane is not simply due to withdrawal of phloem sap: thus, a single insect can cause the early death of the leaf that bears it, while shoot elongation may be checked by very small larvae feeding for only a few days.

When plants of advance growth are attached, the composition of millable canes that survive is abnormal. Analysis of canes where chlorosis of the foliage was the only effect visible to the eye showed that cane quality in the top halves was better and in the bottom halves worse than in comparable portions of unattacked canes (Fig. 4). With canes that clearly showed stunting as well as chlorosis, cane quality in both top and bottom halves was inferior but it was worse at the bottom than at the top. Internal rotting prior to death of canes always began at the bottom and worked upwards.

CONCLUSION

P. iceryi, a species of wide distribution in southern and eastern Africa and in islands of the Western Indian Ocean, is normally innocuous, living on various grasses. Sugarcane is the only cultivated plant on which it has been recorded but this is a very suitable host-plant and the insect, owing to its noxious effect when abundant, is potentially a dangerous pest in countries where it exists in near cane plantations.

In Mauritius and Reunion outbreaks of the insect have occurred at about the same time, indicating that a factor common to the two islands was the cause. Furthermore, the outbreaks have been of short duration. It is believed, therefore, that the weather, not environmental change resulting from agricultural development was in some way basically responsible and that the environment in both islands has been, and is, normally very resistant to population upsurges. It is not to be implied, however, that this situation is permanent.

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LA ESCAMA SUAVE, *PULVINARIA ICERYI* (SIGN.), UNA PLAGA EVENTUAL DE LA CAÑA DE AZUCAR.

J. R. Williams

RESUMEN

Pulvinaria iceryi (Sign.) es una escama o guagua suave (Fam. *Coccidae*) que vive en varias hierbas (zacates) en el Este y el Sur de Africa, y en la parte occidental del Oceano Indico. En las islas Mascarene de Reunión y Mauricio destruyó cañales en 1862-64, y recientemente, en 1977, Mauricio sufrió un segundo brote significativo. Las caracteristicas sobresalientes de la biologia del insecto en 25 hospederos conocidos, se describen en este trabajo. El insecto puede afectar drasticamente la caña de azúcar chupando la savia del floema y posiblemente ejerciendo una acción tóxica despúes de alimentarse. Está considerado como una plaga peligrosa donde quiera que coexista con la caña de azúcar.

THE EFFECTS OF NEMATICIDES AND RAINFALL ON THE POPULATION DENSITIES OF SOIL-BORNE NEMATODES IN FIJI

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ABSTRACT

The use of nematicides in sugarcane had shown reduction in the population densities of nematodes with an increase in sugarcane yield. The two nematicides Temik [2-methyl-2 (Methylthio propionaldehyde 0-methyl carbamoyl) Oxime] and Furadan [2, 3-dihydro-2, 2 dimethyl-7-benzofuranyl-methylcarbamate] were tested at different rates. Temik proved to be superior to Furadan in controlling the soil nematodes. Under natural conditions the population densities of nematodes increase with dry weather and decrease with the rainfall. In Fiji, sugar cane is grown under rainfed conditions, and therefore drier conditions adversely affect the crop.

INTRODUCTION

Helminthological studies were first carried out in Fiji by Cobb[1] on bananas. Serious investigations commenced when it was realized that ginger, bananas and other crops were being affected. Kirby[3] carried out a detailed survey in vegetable crops. With sugarcane, nematode damage was least suspected as the cause of low yield was always attributed to exhaustion of soils, moisture stress, poor fertilization, etc. However, during 1976 inspection of the crop distinct patches of cane suffering from deficiency symptoms were observed. Initial analysis of soil showed dense populations of nematodes. This resulted in a comprehensive survey of all sugarcane soils in Fiji and the evaluation of the effectiveness and economics of chemical control methods.

The survey was conducted over a period of sixteen months to understand the population dynamics and its relationship to soils and other environmental factors. The experiments with nematicides were conducted to assess the economic benefit that may be derived by controlling nematode populations though. Preliminary data was published earlier by Ram Narain and Krishnamurthi[4].

MATERIALS AND METHODS

Nematode infestation survey was conducted by taking 283 soil samples from different locations constituting various soil groups in the sugarcane growing areas in Fiji. These samples were analysed for the presence of parasitic nematodes.

Soil samples were taken with 4.5 cm diameter soil sampling tubes, inserted to a depth to 20-25 cm at a point 5-8 cm from the base of the cane stalks. Six subsamples were taken from each 10-meter plot and after mixing them, 1 kg of sample was taken. Two hundred cc of this soil was used to extract nematodes by means of modified Baeman (Cobb[1]) funnel technique. The extract was made up to 50 ml. Five ml of this extract was taken for counting. During the growing season, observations were made for phytotoxicity and growth responses. Roots were also observed for nematode damage. The cane was harvested after fifteen months of growth. An eighteen-stalk sample from each plot was analysed for biochemical characteristics.

To assess damage caused by nematodes, field trials were planted in known nematode infested areas. Temik, [2-methyl-2 (Methylthiopropionaldehyde 0-methyl carbamoyl) Oxime] and Furadan, [2, 3-dihydro-2, 2, dimethyl-7-benzofuranyl-methylcarbamate] were used at various rates. The trials were planted with commercial variety *Mana* in May, 1977. These were arranged in a randomized complete block design with six replications in the first trial and four replications in the second trial. The plot size consisted of 6 rows by 10 meters with 1.5 meter row spacing.

The nematicides in treatments B to G were applied in granular formulation in the drills at the time of planting. The seven treatments were as follows:

A.	Control	No application
B.	Furadan 3g	75.0 kg/ha
C.	Temik 15g	15.0 kg/ha
D.	Furadan 3g	112.5 kg/ha
E.	Temik 15g	22.5 kg/ha
F.	Furadan 3g	150.0 kg/ha
G.	Temik 15g	30.0 kg/ha

Daily recording of rainfall was made at both the experimental sites.

RESULTS

The percent frequency of occurence of the 13 nematode genera in 283 samples with its distribution over six soil groups are listed in Table 1 (a). The incidence of different types of nematode in cane varieties are listed in Table 1 (b). The population densities of nematodes are shown in Tables 2 (a) and (b).

The fluctuations of soil nematode populations in treatment 'G' and the control in relation to monthly rainfall for the two trial areas are illustrated in Figs. 1(a) and (b).

Nematode species belonging to several different genera were common in extracts of soil from different soil groups and root samples. These nematodes, listed in possible order of decreasing significance on cane in Fiji are *Pratylenchus, Helicotylenchus, Tylenchorhynehus, Criconemoides, Hemicriconemoides, Rorytenchulus, Meloidogyne, Trichodurus, Hoplolaimus,* and *Radopholus.*

The different genera are correlated well with specific soil groups, e.g. *Meloidogyne* is present mostly in sandy soils whereas *Pratylenchus* has high population densities in most other soil groups. Humic latosal soil group had lower population densities compared to others.

Evidence of root damage to young plant crop was widespread. Damage took the form of distortions, lesions, necrosis, stubby roots, root galls, and general unthriftiness. Root damage in mature plant and ratoon crops was difficult to asses.

TABLE 1 (a). Occurence of nematodes in different soil groups.

	Genera	Percent+ A	B	Occurence in soil group* C	D	E	F
1.	*Pratylenchus*	45	65	82	82	76	88
2.	*Helicotylenchus*	34	76	87	62	68	80
3	*Tylenchorhynchus*	45	18	33	69	39	61
4.	*Criconemoides*	44	41	40	38	25	34
5.	*Hemicriconemoides*	22	29	34	51	28	42
6.	*Meloidogyne*	44	18	24	13	11	8
7.	*Rotylenchus*	10	41	21	21	29	16
8.	*Xiphinema*	20	0	6	23	7	7
9.	*Trichodorus*	22	6	12	8	1	8
10.	*Hoplolaimus*	33	0	10	21	4	4
11.	*Radopholus*	21	0	6	5	8	0
12.	*Heterodorus*	33	0	0	3	0	0
13.	*Paratylenchus*	0	0	0	3	0	0

**Soil groups given above are as follows:*
A = Sandy soils
B = Humic Lalosols
C = Ferrigeneus Lalosols
D = Alluvial soils
E = Nigresecent soils
F = Gley soils

$$+ = \frac{\text{No. of samples with various nematode genus} \times 100}{\text{Total No. of samples from that specific soil type.}}$$

TABLE 1(b). Incidence of different types of nematode in cane varieties.

Variety	Total No. of Samples	Types of Nematodes present in the number of samples+													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ragnar	187	167	102	39	113	76	11	12	38	24	14	50	10	8	2
Mali	95	78	29	20	48	42	8	11	25	4	4	20	2	3	1
Waya	80	64	27	29	47	35	5	16	13	11	10	19	7	8	1
Mana	19	16	6	3	13	15	2	3	6	3	2	4	–	–	–
LF55-8283	5	3	3	1	2	3	2	1	1	–	–	1	1	–	–
Ono	4	3	–	1	4	–	–	–	–	–	–	–	–	–	–

1. *Pratylenchus*
2. *Tylenchorynchus*
3. *Meloidogyne*
4. *Helicotylenchus*
5. *Criconemoides*
6. *Trichodorus*
7. *Radopholus*
8. *Rotylenchus*
9. *Xiphinema*
10. *Hoplolaimus*
11. *Hemicriconemoides*
12. *Paralongiderus*
13. *Paratylenchus*
14. *Heterodera*

DISCUSSION

From the data it is obvious that the nematode population differed from one treatment to another. As the rate of nematicides increased the population density decreased obviously revealing the effect of chemicals used. Throughout the sampling period the treatment G having Temik at the highest rate (30.0 kg/ha) had lowest population density and yielded more cane (Table 3).

The monthly counts showed that the nematode population fluctuates with the weather, especially rainfall (Table 4). The population increased with the dry weather and decreased with rainfall precipitation. Fig. 1(a) and (b) show that 10 days after the rainfall the population decreased steeply. This indicated that during a year when there was uniformly distributed rainfall the nematode effect was not visible.

The effect is pronounced only when there is a prolonged drought. Under such conditions *Pratylenchus* and *Tylenchorhyncus* damage the roots considerably. It is fortunate that the authors have been unconciously testing the varieties on wide range of locations with the resulting bulk of the varieties tolerant to nematodes. The varieties also recovered very fast once the weather conditions are favourable.

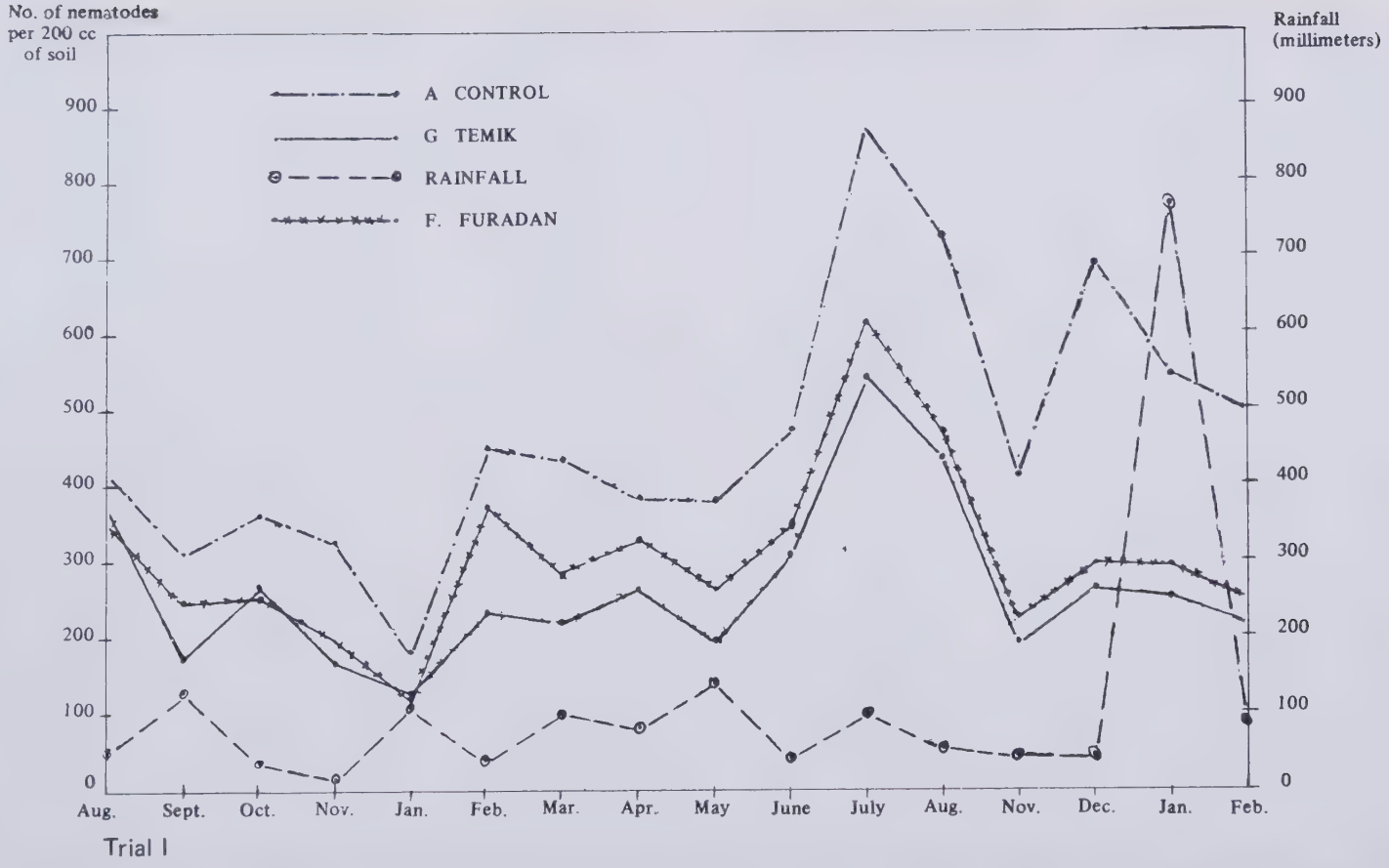
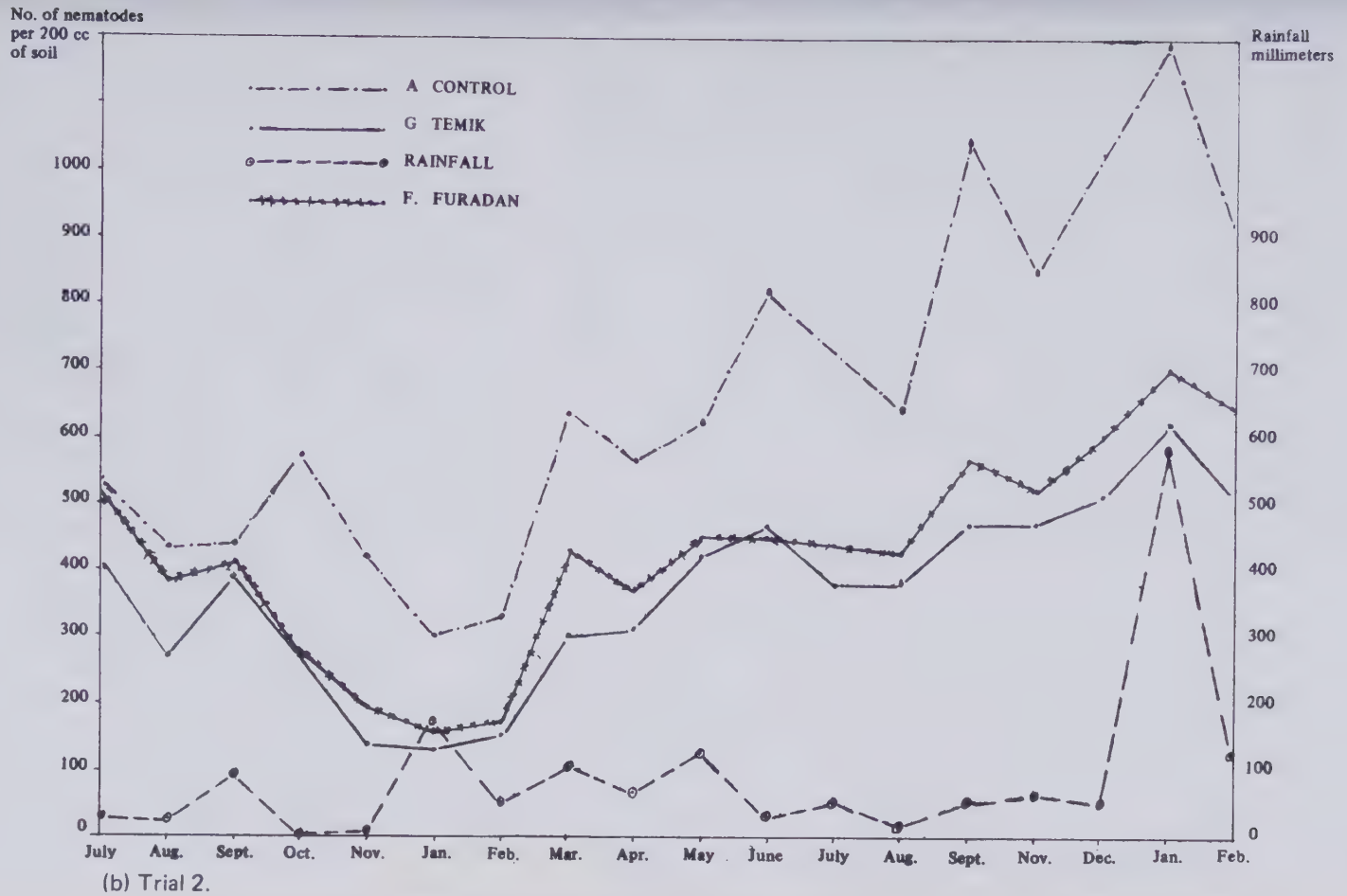


FIGURE 1a. Fluctuations in nematodes populations in relation to rainfall in cane plots tested with different nematicides.



(b) Trial 2.

FIGURE 1b. Fluctuations in nematodes populations in relation to rainfall in cane plots tested with different nematicides.

TABLE 2(a). Trial 1. Population densities of nematodes per treatment

Date sampled	Nematicide treatments*						
	A +	B +	C +	D +	E +	F +	G +
11. 8.77	415	379	298	337	273	257	369
12. 9.77	311	274	231	211	238	249	177
12.10.77	361	248	359	234	243	258	264
18.11.77	326	268	229	200	170	195	166
29. 1.78	182	140	166	114	129	120	125
28. 2.78	450	508	295	347	394	376	237
20. 3.78	436	306	254	302	258	283	220
28. 4.78	386	370	240	261	335	327	261
19. 5.78	380	306	290	309	273	260	195
6. 6.78	4.75	411	446	411	441	346	306
7. 7.78	868	858	661	603	641	615	540
1. 8.78	723	583	463	528	451	470	436
3.11.78	411	381	316	271	233	223	193
11.12.78	695	523	441	350	391	298	261
15. 1.79	540	488	470	410	393	293	256
6. 2.79	498	436	385	365	363	251	215

** Mean of 6 replications*

+ Codes as in Materials and Methods.

It is also suspected that many herbicides particularly 2, 4-D and 2, 4, 5-T killed most of the soil invertebrates except the nematodes. This may aggravate the situation. This aspect is under further investigations.

The nematicides had no effect on germination nor had any phytotoxic effects on the cane plants. At an early stage the cane showed slight differences in growth but as the drought set in the differences were no longer visible. The stooling was relatively better in treatments E, F and G. Root systems showed symptoms of varying degrees of damage i.e. the treatment 'G' had very few infected roots.

The harvest results showed that with the increase in the rate of nematicides the yield increased. Treatment G gave higher yield compared to other treatments. There was a difference in yield of 8.8 tons/ha in trial 1 and 15.9 tons/ha in trial 2 between the control and the treatment G.

At the time of harvesting, eighteen stalk samples were taken from every plot and analysed for sucrose, fiber starch and purity. There was no significant difference between the control and the treated plots. The namaticides had no adverse effects

TABLE 2(b). Population densities of nematodes per treatment

Date sampled	Nematicide treatments* A +	B +	C +	D +	E +	F +	G +
25. 7.77	536	410	281	420	379	517	408
17. 8.77	434	377	422	421	282	382	270
23.9.77	438	398	389	385	336	417	392
25.10.77	571	463	414	345	322	275	274
29.11.77	421	370	333	275	200	196	139
16. 1.78	308	256	237	266	182	163	134
15. 2.78	329	244	159	172	153	174	151
8. 3.78	639	396	438	540	357	434	304
13. 4.78	567	594	427	367	390	375	313
12. 5.78	623	495	540	468	503	445	423
28. 6.78	820	803	750	688	655	455	467
21. 7.78	725	540	500	475	485	440	382
16. 8.78	643	515	555	485	532	425	385
22. 9.78	1045	755	640	652	612	565	475
24.11.78	850	787	710	660	615	522	477
15.12.78	1027	945	890	857	800	607	515
26. 1.79	1192	1197	1042	975	907	705	625
19. 2.79	921	880	877	842	775	650	510

** Mean of 6 replications.*

+ Codes as in Materials and Methods.

TABLE 3. Harvest Results

Replication	Treatments—Yield in kg/plot A	B	C	D	E	F	G
Trial 1							
1	272	334	256	265	366	260	321
2	235	231	304	325	298	243	274
3	243	271	309	258	241	225	416
4	314	249	210	282	268	297	273
5	260	289	328	296	329	321	324
6	241	242	296	258	240	229	296
Ave.	261	269	284	281	290	262	317
Tons/ha	42.1	43.3	45.6	45.1	46.6	42.1	50.9

Table 3. (cont.)

Trial 2							
1	94	136	154	227	194	130	203
2	124	121	217	175	198	210	216
3	121	213	147	235	116	163	277
4	144	188	236	189	204	178	178
Ave.	121	164	189	207	178	170	219
Tons/ha	19.4	26.5	30.2	33.3	28.7	27.5	35.3

TABLE 4. Monthly rainfall recording (millimeters)

Month	Trial 1	Trial 2
August 1977	50.5	27.0
September	128.6	91.0
October	38.5	0.5
November	13.4	3.8
December	66.5	87.9
January 1978	102.3	176.7
February	39.1	54.7
March	99.2	106.7
April	80.7	67 5
May	138.5	127.5
June	41.0	30.5
July	99.5	57 2
August	58.6	18.5
September	43.6	51.6
October	183.2	97.8
November	41.9	66.0
December	40.1	57.0
January 1979	764.8	582.9
February 1979	84.6	122.0

on the biochemical characters and thus did not affect the quality of sugar. No residue analysis was done.

From the preliminary investigations it is evident that if Temik is used at 30 kg per ha it would increase the yields substantially. However, this would be true only in relatively dry year. Therefore the rate and time of application is under review so that application proves economical.

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EFECTOS DE LOS NEMATICIDAS Y LAS LLUVIAS EN LA POBLACION DE NEMATODOS DEL SUELO EN FIJI.

R. Narain and M. Krishnamurthi

RESUMEN

El uso de nematicidas en caña de azucar redujo la poblacion de nematodos aumentando el rendimiento en caña. Dos nematicidas fueron probados a distintas dosis: Temik* y Furadan**. El primero demostro ser superior al Furadan para el control de los nematodos del suelo. Bajo condiciones naturales las poblaciones de nematodos aumentan durante la sequia, disminuyendo en la epoca de las lluvias. En Fiji la produccion de caña esta supeditada al regimen de lluvias, y consecuentemente una seca prolongada afecta significativamente la produccion.

* *Conocido tambien como Aldicarb y Carbanolate.*
** *Conocido tambien como Carbofuran y Curaterr*

FOLIAR APPLICATIONS OF OXAMYL TO RATOON SUGARCANE FOR NEMATODE CONTROL

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ABSTRACT

The harvest results of seven field trials using foliar spray applications of the systemic nematicide oxamyl (Methyl N', N'-dimethyl -N-(methylcarbamoyl) oxyl-1-thiooxamimidate) to ratoon sugarcane are presented. Single and repeated split sprays were applied by knapsnack to give an applied dose of 3.0 kg a.i./ha to ratoon regrowth 6-8 weeks after harvest.

Good responses averaging an additional 2.5 tons sucrose per hectare were obtained and these were equal to responses from conventional soil-applied applications of aldicarb granules. The many advantages of using a foliar-applied nematicide on steep erodible hillslopes, and where trash and leaf residues are retained as a mulch, are discussed.

INTRODUCTION

Yield losses due to nematodes are often considerable in sandy soils and the use of nematicides has become well-established within the South African sugarcane industry. The nematicides most commonly used have been aldicarb and carbofuran, but these are soil-applied and require the subsurface placement of granules. This method ensures the best result and also reduces toxicity dangers to stock and wildlife.

Subsurface granule application is easily done at planting time when the granules may be dropped into the planting furrow before it is closed. Treatment of the ratoon crop (stubble cane) involves the opening of a 5-7 cm deep furrow beside each row. This operation is easily carried out on burnt fields but is more difficult to achieve when the trash and leaf residues are retained as a natural mulch. The trash has to be either parted manually or a specially designed tractor-powered machine has to "saw through" the trash blanket[1]. The nematicide granule is then dropped into the cut slit. Unfortunately the machines are costly to manufacture and operate and consequently their use has been limited.

Trashing is recommended for steep hillsides and on the average a response

of 9 tons cane per hectare per annum can be expected from retaining the trash mulch (Thompson[2]). There are also longer-term benefits to be obtained from reducing soil erosion.

There is thus a need for a systemic nematicide that can be foliar-applied to the crop, and with the development of such a treatment knapsnack applications would become possible in those areas where, for reasons of terrain, tractor-powered equipment cannot be used. The nematicide oxamyl has good systemic activity and has been reported as being translocated downwards to the roots and rhizosphere (du Pont de Nemours and Co.[3]). It is active on a wide range of nematode species found in Southern Africa (Harris[4]; Milne[5]). A 24% liquid formulation is available commercially as du Pont Vydate L.

Replicated field experiments were conducted to evaluate oxamyl against aldicarb, the only soil-applied nematicide then being used in the sugar industry. This paper presents some of the results of the ratoon trials treated during 1976 and 1977.

EXPERIMENTAL PROCEDURES

The field trials were conducted on a number of sugar estates in South Africa. The physical characteristics of the soils on the experiment sites are presented in Table 1, which also includes information about the respective sugarcane crops.

Field layouts were generally of a randomized block design with treatments being replicated four times. The plots consisted of five cane rows each 10 meters long, and the area treated with each chemical treatment amounted to 0.024 hectare per experiment. Although initial land preparation varied from trial to trial, the application of treatments and the methods of assessment were similar.

Sites were selected because of the visual presence of nematode-damaged roots

TABLE 1. Details of experimental sites

Trial	Soil series	pH	Clay%	O.M.%	C.E.C. (% m. eq.)	Ratoon	Variety
1	Clansthal	7.6	6	1.1	5.0	1st	N55/805
2	Fernwood	5.1	5	0.5	3.3	2nd	NCo376
3	Fernwood	6.0	4	0.4	2.6	1st	N55/805
4	Fernwood	6.0	4	0.4	2.6	2nd	N55/805
5	Clansthal	5.8	3	0.5	3.3	1st	N55/805
6	Clansthal	6.9	5	0.8	3.2	4th	NCo376
7	Fernwood	6.0	3	0.5	4.2	1st	N55/805
8	Fernwood	5.7	5	0.7	3.1	1st	N55/805

in previous crops and also for a known inability to produce satisfactory crops. Because of these criteria and the generally low fertility of the soils, there was a high natural variability within each site.

The sugarcane crop was usually a first or second ratoon of variety N55/805. This variety has proved fairly popular for use in sandy soils where nematodes have contributed to poor yields. However, unless nematicides are used it is still rare for acceptable yields to be harvested from third and fourth ratoon crops.

The aldicarb granular treatments were applied into a single hand-drawn furrow on one side of each cane row by means of a Sequip microband applicator. The standard dose of aldicarb at 3.0 kg. a.i./ha was applied in the form of 20 kg/ha product containing 15% active material.

The foliar oxamyl treatments were applied by means of a Cooper Peglar CP_3 knapsack fitted with a Delavan 33974-2 Raindrop nozzle delivering 300 liters per hectare at a spraying pressure of 100 Kpa. Oxamyl doses varied from 3 to 20 l/ha (as the 24 % formulation Vydate L) in single and multiple split applications.

The sprays were applied without any additional adjuvants and were directed over the center of the cane rows. The crop canopy was about 50-60 cm high with 6-8 leaves unfurled per shoot, the crop being about 6-8 weeks old following the previous harvest.

Effects on the crop were assessed at regular intervals. The heights of ten randomly selected shoots per plot were measured from ground level to the top visible dewlap. The sugarcane yields were measured at harvest and the yield of sucrose determined. Residue analyses were done on plant material collected at varying time intervals after treatment.

RESULTS

The foliar sprays of oxamyl usually gave satisfactory yield responses and there were only small differences between the yields of cane which had been treated with aldicarb or oxamyl. The 12 *l*/ha dose of oxamyl, whether in a single application or as an 8 + 4 *l*/ha repeated split application 6 weeks apart, was consistently the most reliable. The yield results for this dose are listed in Tables 2 and 3. For the ratoon trials treated during 1976 and 1977 a mean additional response of 17.4 tons cane per hectare was obtained. After milling this represented a gain of 2.5 tons sucrose per hectare over the untreated control.

Responses were relatively smaller where the untreated crop was already growing fairly well. Then either the growing conditions or the nematodes markedly affected the untreated crop the response to foliar oxamyl increased the cane yield by as much as 75%.

TABLE 2. Mean yields (tons cane/hectare) for oxamyl and aldicarb treatments applied at 3 kg a.i./ha. (Oxamyl applied as 12 l Vydate L 24%/ha)

Trial	Date of 1st spray	Oxamyl dose as Vydate L(*l*/ha)	Yield of Untreated Control (tc/ha)	Yield response over control (tc/ha)	
				oxamyl	aldicarb
1	7. 1.76	6 + 6	94	+ 8	+ 12
2	16. 1.76	6 + 6	57	+ 21*	+ 15
3	30. 1.76	6 + 6	42	+ 32*	+ 25*
4	29. 9.76	8 + 4	64	+ 33*	+ 25
5	17.11.76	8 + 4	67	+ 13	+ 20*
6	18.11.76	8 + 4	81	+ 7	+ 20*
7	21.11.76	8 + 4	70	+ 14*	+ 9
7	21.11.76	12	70	+ 11*	+ 9
		Mean Yield	68	+ 17.4	+ 17.4

** statistically significant (P > 0.05)*

TABLE 3. Mean yields (tons sucruse/hectare) for oxamyl and aldicarb treatments applied at 3 kg a.i./ha. (Oxamyl applied as 12 l Vydates L 24%/ha)

Trial	Date of 1st spray	Oxamyl dose as Vydate L(*l*/ha)	Yield of untreated control ts/ha	Yield response over control ts/ha	
				oxamyl	aldicarb
1	7. 1.76	6 + 6	12.8	+ 1.1	+ 2.1
2	16. 1.76	6 + 6	6.6	+ 3.4*	+ 2.7*
3	30. 1.76	6 + 6	6.3	+ 4.8*	+ 3.9*
4	29. 9.76	8 + 4	9.0	+ 3.9	+ 2.8
5	17.11.76	8 + 4	8.5	+ 1.9	+ 2.0
6	18.11.76	8 + 4	11.0	+ 0.8	+ 3.1*
7	21.11.76	8 + 4	11.1	+ 2.2*	+ 1.4
7	21.11.76	12	11.1	+ 1.9*	+ 1.4
	Mean Yield		9.6	+ 2.5	+ 2.4

** statistically significant (P > 0.05)*

Acceptable results were also obtained from oxamyl treatments used at lower doses. However the high natural variability of the sites made it difficult to accurately select the optimum dose. Fig. 1 displays sucrose yields from the treatments in Trial VII which was established on a site of above average uniformity. It will be seen that the differences between yields obtained due to different oxamyl treatments were not large within the 8 to 12 l/ha dose range. Split applications often gave slightyly better results, but the small additional responses would not normally justify the extra cost and supervision needed for the second spray

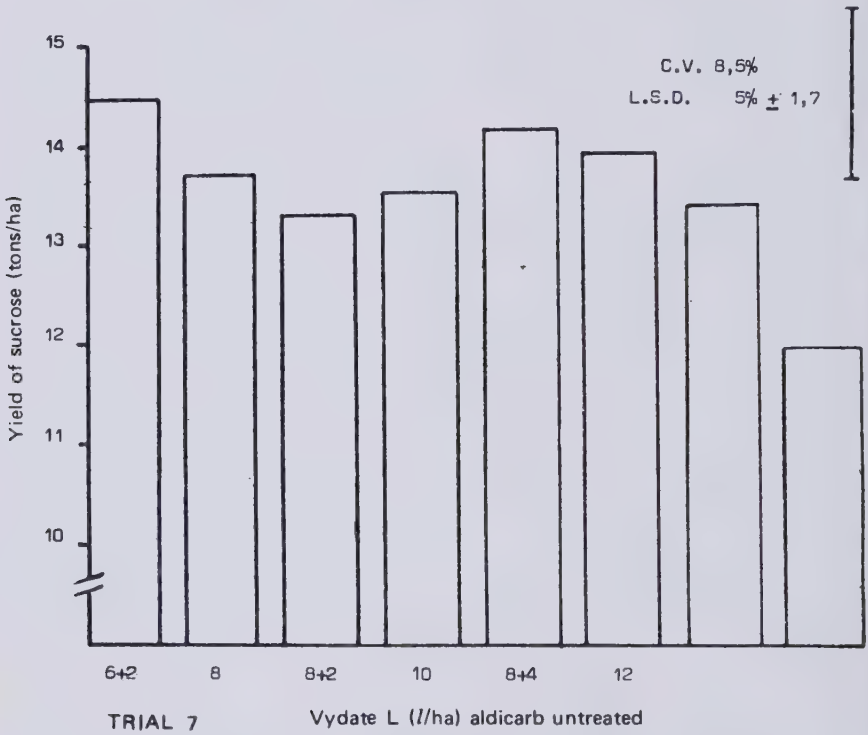

FIGURE 1. Sucrose yields at harvest

Most experiments were initiated when conditions suitable for active growth were present during the spring and summer. A more recent trial was sprayed in July 1978 during cold dry winter conditions. The mean shoot height responses are encouraging and are presented in Fig. 2.

Oxamyl residues were studied in two additional trials. Analyses showed rapidly declining residues after application. In one of the trials acceptable residue levels (less than 0.05 ppm) were obtained after 32 days. Sugarcane in Southern Africa is not normally harvested less than 300 days after a foliar spray might be applied.

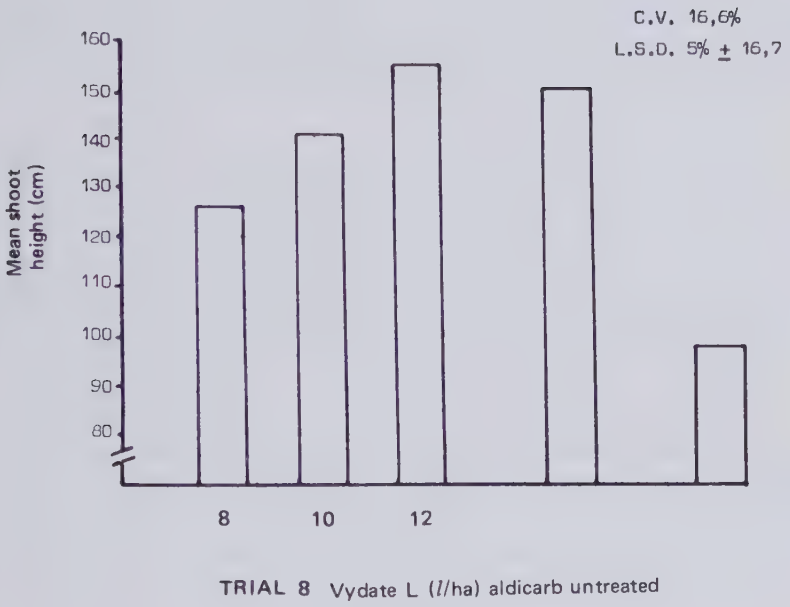

FIGURE 2. Mean shoot heights 7 months after application of single doses of oxamyl and aldicarb.

DISCUSSIONS

An earlier series of trials in 1973 and 1975 evaluated foliar oxamyl on plant crops (Harris[4], TFO[6]). Compared with the use of subsurface granular nematicides at planting the responses were unsatisfactory and investigations were discontinued. It is suggested that the main reasons for poor responses in plant crops were a low leaf-to-root ratio for nematicide absorption when the sprays were applied, and because the rapidly developing young roots remained unprotected from nematodes for too long a period. In a newly cut ratoon crop the rapidly unfurling leaves present a high leaf-to-root ratio which is enhanced by a delay in establishing the new ratoon root system.

In addition to the replicated trials 300 hectares of ratoon sugarcane on some 20 different farms were sprayed during 1978 with a single dose of 12 l Vydate L/ha. Although not yet harvested the visual responses have been good and the growers concerned have expressed satisfaction with the treatments. In addition to usage on hillslopes and where trash mulching is practiced other benefits have been reported. Foliar nematicides have additional potential on flatter and less broken land because of the ease of application through standard farm sprayers. Considerable savings in fuel and time can be made because a single tractor swathe can treat

up to six cane rows at a time, whereas the subsurface placement of granules is limited to a maximum of one or two rows. The selected spot spraying of weaker areas within a field is possible, and foliar sprays can also be used on odd-shaped fields and where drainage ditches and short cane lines are present. Soil disturbance and root damage is reduced to a minimum.

Further work is required to compare the efficacy of foliar-applied and granular nematicides under the different, and particularly extreme, environmental conditions of low and high rainfall. Poor results might be expected from the use of a foliar nematicide if rain follows before there has been adequate translocation of active material into the leaf tissue. In the field experiments no account was taken of the nematode species composition within the sites and of the different times of application, all of which are factors that require additional investigation.

Oxamyl is a potent anticholinesterase agent that is highly toxic. The inhalation dangers cannot be underestimated and it is essential that full protective clothing and masks be used whenever the product is applied. Inhalation risks are considerably reduced by the use of low pressure equipment with spray pressures of 100 Kpa. and by the use of Delavan 33974-2 Raindrop nozzles or other spray nozzles that produce large droplets and minimize drift.

CONCLUSIONS

Water-based foliar sprays of oxamyl at about 3.0 kg i.a./ha (applied as 12 ℓ Vydate L24%/ha) have proved equal in efficacy to aldicarb granules (3.0 kg a.i./ha) applied below the soil surface. Under variable field conditions both nematicides produced good yield responses amounting to an extra 2.5 tons sucrose per hectare over the untreated cane. From the results so far obtained oxamyl should be sprayed when the ratoon crop is at the 6-8 leaf stage, which is usually about 6-8 weeks after the previous harvest provided that good crop tillering has taken place.

Oxamyl sprays can be recommended for use on ratoons but not on plant crops. They have potential for use on hillslopes, where trash mulching is practiced, and also in many field situations were granular nematicides cannot readily be applied. Although there is no residue danger from oxamyl in the crop, it is nevertheless a highly toxic product and extreme care must be taken when it is being sprayed. Conventional knapsnack and tractor-powered equipment may be used but low drift nozzles and low spraying pressures are recommended to minimize chemical drift and inhalation.

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APLICACION FOLIAR DE OXAMYL* A RETOÑOS PARA EL CONTROL DE NEMATODOS EN CAÑA DE AZUCAR.

F. E. Richardson y E. J. Watt

RESUMEN

Se presentan los resultados de siete pruebas de campo usando aplicaciones foliares del insecticida sistemico Oxamyl (Metil N', N'-dimetil-N-[(metilcarbamoyl) -1-thio oxamimidate] a retoños de caña de azucar. Se realizaron rociaduras sencillas y fraccionadas con mochilas manuales a retoños de 7 a 8 semanas, con dósis de 3 kilogramos por hectarea cada una.

Los resultados obtenidos fueron tan satisfactorios como los de tratamientos convencionales al suelo usando Aldicarb** granulado, promediando unas 2.5 toneladas mas de sacarosa por hectares sobre los testigos. Se detallan en este trabaho las ventajas de usar un rociador foliar en las laderas muy pendientes susceptibles a la erosion, y donde las hojas y cogollos secos de caña se dejan en el campo como coberteras.

* *Vydate*
** *Temik-Carbalonate*